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## New applications of disease genetics and pharmacogenetics to drug development

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TOMMORROW is a Phase III delay of onset clinical trial to determine whether low doses of pioglitazone, a molecule that induces mitochondrial doubling, delays the onset of MCI-AD in normal subjects treated with low dose compared to placebo. BOLD imaging studies in rodents and man were used to find the dose that increases oxygen consumption at central regions of the brain in higher proportion than activation of large cortical regions. The trial is made practical by the use of a pharmacogenetic algorithm based on TOMM40 and APOE genotypes and age to identify normal subjects at high risk of MCI-AD between the ages of 65–83 years within a five year follow-up period.

### Addresses

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It is important when discussing genetics and pharmacogenetics [PGX] in clinical pharmacology and drug development to acknowledge the technologies that are commonly practiced in drug development. From a genetics point of view, the contents of clinical pharmacology databases for drug metabolites and PK/PD have certainly been extended by genetic/genomic technologies over the past two decades. In particular, sequencing

of highly polymorphic loci such as the HLA region and genes involved in drug metabolism have increased specificity of genetic associations with pharmacokinetic or pharmacodynamic phenotypes. This expanded compendium of polymorphisms, including less frequent polymorphisms, has increased our ability to explore the genetics of uncommon events in different ethnic groups. One example is the genetic underpinnings of the hypersensitivity syndrome observed in about 4% of Caucasian users of abacavir to treat HIV infection [1]. GlaxoWellcome received an accelerated approval to market abacavir, but approval was accompanied by an expectation by the FDA and EMA that the company would investigate the hypersensitivity syndrome. Cooperation throughout clinical development at GlaxoWellcome was necessary to undertake the experiments that would allow for the identification of the subgroup of patients for whom abacavir was contraindicated. While the HLA-B57 locus was known in 1997, from a database point of view it took several years to identify the B-5701, 5702 and 5703 polymorphisms at the locus using genomic sequencing. The risk of the hypersensitivity reaction was present only in patients with the B-5701 allele.

Today human drug trials provide opportunities to identify efficacy responders and to characterize their genetics compared to non-responders [2]. Since very large numbers of patients are usually not available [affordable or practical] as they may be for studies of risk factors for common diseases studies, the analytical process is a bit reversed from searching for genetic mutations for Mendelian disease diagnoses. Patients must be identified and followed during and after treatment. Predicting efficacy for individual patients who might also be at risk for an adverse response will take time to become common in medicine. Drug trials take years and must be designed to prospectively evaluate patient responses. A basic requirement for genetic testing is that consented DNA samples must be collected across the entire study. In reality, to accomplish collection of DNA from all study participants requires planning and patient consent, without which pharmacogenetics cannot be used effectively for hypothesis generation, hypothesis testing and for regulatory decisions.

It is a bit different for uncommon adverse events where the genetic loci associated with relatively rare, overt

phenotypes are typically easier to identify. For safety PGX, the uncommon mutations can be assessed against population samples, but for efficacy within a trial it is necessary to study all participants so that efficacy and lack of efficacy can be compared in participants who received drug. It is, however, a problem when DNA is not collected or unavailable in individuals with adverse events. Banking DNA samples from important studies is still quite uncommon in the industry [1,14].

The goal of efficacy PGX is to find a genetic biomarker that predicts high expectation of efficacy in a sufficient number of people to qualify the test for selection of drug therapy. These studies focus on the response to treatment, and may be quite distinct from genetic diagnostic tests for disease. Beginning with any early Phase II treatment trial, the objective is to differentiate those treated individuals who respond to the drug but are not responsive to placebo. This can initially be evaluated in small studies during which defined clinical responses are measured. The more obvious the efficacy response, the more certain early associations can be made. Efficacy PGX depends on the choice of therapeutic agent, dose, and the responses of the individuals receiving the drug. It is also influenced by the type of genetic search performed. Candidate gene lists involving proposed disease pathways have provided an effective starting point for translation to clinical trials for several decades. Genome-wide association studies (GWAS) have produced many gene lists with subsequent rationales provided for selected genes. To date, there are few examples where specific genes “discovered” by GWAS lists have translated into clinically relevant programs, outside of oncology where affected tissue is more available to confirm with gene expression studies.

Each subsequent Phase II trial will provide greater statistical support for markers associated with drug efficacy. Recently, an investigation of highly polymorphic structural genes that carry highly polymorphic variations at a single locus compared to SNPs has reduced the time necessary for translation to clinical trials [3]. A high degree of polymorphic variations contribute to the proportion of individuals in a population who are informative at that locus. Efficacy biomarkers identified and validated in Phase II may be useful in proof of concept studies and to stratify and improve the efficiency of Phase III trials. PGX markers that enrich for responders in Phase III studies may allow for a more robust efficacy signal and a faster path to registration. Companion diagnostics can be also be qualified in late clinical trials to identify individuals with a higher ‘risk’ of a beneficial response [3,4]. It should also be noted that polymorphisms in one ethnic group may be absent or occur at a lower allele frequency in other ethnic groups. This emphasizes the need to examine genetic markers carefully in different ethnicities, especially with highly variable markers [5].

This short opinion piece will emphasize some of the newer genetic technologies that can facilitate Phase III trial design. Efficacy PGX is a relatively new application of genetics in clinical development because it requires DNA collection during the trial. In this new paradigm for drug development, drug discovery becomes more dependent on studies that utilize these PGX associations.

Safety PGX in the aforementioned abacavir trial demonstrates the translation of a genetic marker to ensure safer use of the medication. In addition, economics are often the major consideration for use of a test, particularly for defining efficacy for reimbursement. Predicting adverse responses also leads to extended market use in addition to enhanced safety long after patent exclusivity for the drug compound expires. In the case of abacavir it is still used in HIV drug combinations for HLA-B5701 negative patients, thereby extending its commercial value after its chemical patents expired.

### **New technologies and innovation in a Phase III clinical trial: trial design to study delay of onset**

Central nervous system [CNS] diseases have recently experienced declining interest by major drug developers because the costs of doing clinical studies in these disease areas can be high and likelihood of approval low. The basic premise that early clinical pharmacology and safety studies can rule out [kill fast] compounds works for Phase I, but there are major differences in the cost of Phase II and Phase III clinical studies. It can be very costly to run clinical trials to observe efficacy in a dose-finding trial. This is especially true for longer trials with clinical endpoints followed over months, such as those performed for neuropsychiatric disorders.

Studying clinical pharmacology in animals and translating these findings into human studies is critical for generating information on dose and trial design that can translate into reducing overall drug development cost and time to approval. Imaging technologies have now been applied to measure effects of different drugs and drug doses on the pharmacodynamic response of specific anatomical areas in the brain. While drug dosages are usually pushed to the highest tolerated level to ensure that efficacy will not be missed, the chances for adverse events are raised as well. For neurological diseases, imaging studies in animals provide a way to determine the most reasonable, effective dose without waiting for expensive clinical efficacy studies to define it.

PET and fMRI imaging studies in animals and man can now be used for dose finding studies in late discovery and early development, particularly in the neuropsychiatric diseases from which major pharmaceutical companies have generally withdrawn over the past five years. These data can quickly and economically define the lowest dose

with activity in specific regions of the brain, allowing dose-finding studies to suggest lower, and generally safer, brain-active doses in a shorter period of time. This is particularly relevant to disease prevention studies, where a drug is used to treat unaffected people at high risk of developing a particular disease. Dose selection for the drug that is being used in a delay of onset of Mild Cognitive Impairment due to Alzheimer's Disease [MCI-AD] trial, the TOMMORROW study, was informed by a BOLD MRI study.

### The genetic, imaging and phylogenetic mapping basis for translation to the TOMMORROW Phase III clinical trial

The TOMMORROW clinical trial, a large, Phase III trial to assess delay of onset of MCI-AD in cognitively normal subjects between 65 and 83 years of age at entry, was designed to qualify a predictive genetic biomarker in the context of delaying the onset of MCI-AD using a drug that may work by increasing the number of mitochondria in cells. Animal BOLD imaging, which measures anatomically specific oxygen utilization during cerebrovascular response, was used to identify the lowest dose of pioglitazone that would activate metabolism in the central areas of the brain that are associated in humans with clinical AD [34\*\*] (Figure 1).

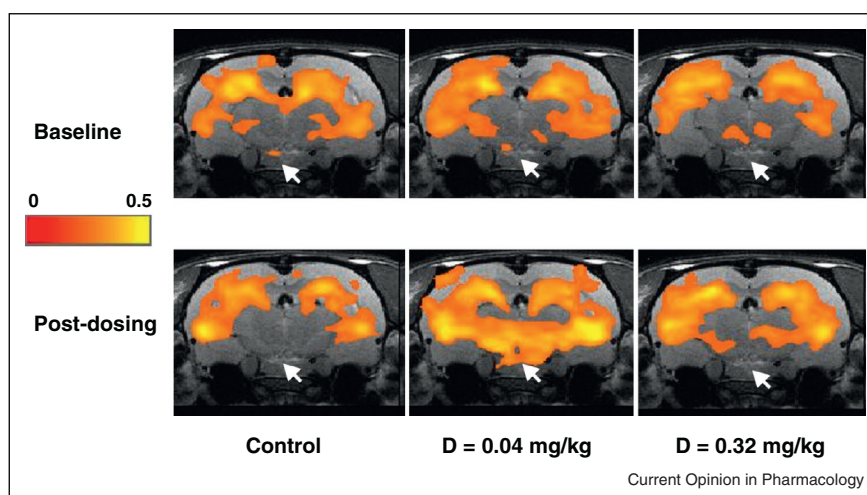
In order to translate these animal model data to humans, functional BOLD imaging was conducted following a memory test in people who received 14 days of pioglitazone treatment. BOLD fMRI responses were used for dose finding studies of pioglitazone using a within-subject BOLD fMRI paradigm targeting episodic memory-related hippocampal activation (Figure 2).

These BOLD data led to the choice of drug dosage of 0.8 mg/kg for the Phase III TOMMORROW study. The TOMMORROW trial is designed to qualify the genetic biomarker, an algorithm composed of TOMM40 and APOE genotypes and age at study entry, used to identify those at high risk to develop MCI-AD and simultaneously test the efficacy of pioglitazone to delay the onset of this condition in high risk subjects. (Delay of onset trials are frequently referred to as prevention trials, but to establish prevention of the condition would require a much longer study than the 5-year treatment period that is anticipated.) This daily dosage is less than 3% of the dosage approved for the treatment of type 2 diabetes mellitus. Pioglitazone boasts a well-documented tolerability record of more than 22 million man-years of clinical use at the doses marketed for treatment of type 2 diabetes mellitus (15–45 mg daily). A lower dose would seem to be safer for use in normal individuals whose TOMM40 and APOE genotypes, and age would suggest a higher risk of MCI in the next five years.

### Other genetic technologies

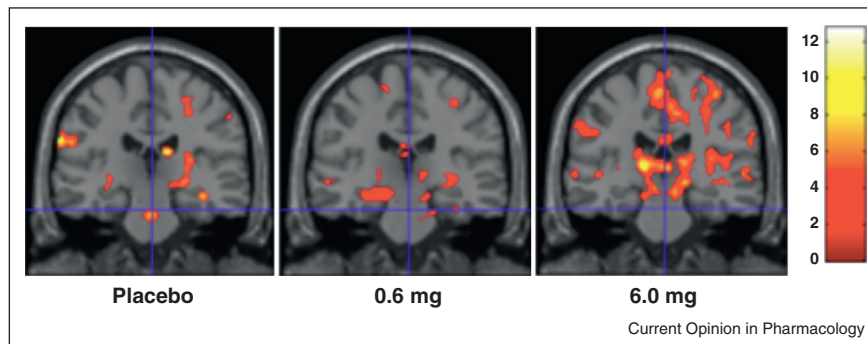
It is important to delineate the other genetic technologies that led to the TOMM40, APOE, and age-at-entry algorithm that is being qualified. Several years ago, we suggested using molecular evolutionary analysis [phylogenetic mapping] to describe the complex genetics of neurodegenerative diseases that have a component of inherited risk to identify clinically useful biomarkers and disease-associated variants. Across the many GWAS studies for complex diseases, there were floods of newly identified 'disease genes' with small to moderate (odds ratio of 1.1–2.0) effect sizes. Still, several years later, many of the variants identified by GWAS have not been

Figure 1



An illustration of the experimental results reported in a dose finding experiment in awake rats using resting state BOLD MRI. Increased functional connectivity between the CA1 region of the hippocampus and the ventral thalamus and hypothalamus is seen following 7 daily doses with 0.04 mg/kg of pioglitazone, far below the dose usually recommended to treat diabetes mellitus [30–45 mg daily]. Note that the dose of 0.32 mg/kg, which is still well below human doses for diabetes mellitus, has diminished central activity.

Figure 2



An illustration of the results of the human fMRI experiment, demonstrating the activation of oxygen utilization in central regions of the brain usually affected later by AD. Using this within-subject pharmacologic BOLD fMRI paradigm while testing episodic memory hippocampal activation in the left hippocampal area was observed, again at a very low dose. At higher pioglitazone doses there is more cortical activity present and less hippocampal activation observed (Zeineh *et al.*, Science 2003).

translated into actionable findings for disease risk prediction or PGX [6,7<sup>\*\*</sup>,8<sup>\*\*</sup>].

Association of APOE4 with increased risk and earlier age of onset of late-onset AD was first reported in 1993 [9,10<sup>\*\*</sup>]. At this time, the association was widely criticized by AD genetic experts in national and international meetings, sometimes with presented data later found to be technically in error. Published reports of relatively small clinical studies confirmed the association between APOE genotype and risk of AD [11–16]. Since this time, multiple GWAS have confirmed that the region containing APOE is highly significantly associated with risk of developing AD; spectacular *p* values were reported for the association of TOMM40 SNPs within the LD region containing the APOE gene, for example, in the study of Harold *et al.* [17] with four TOMM40 SNPs [including rs2075650,  $P = 1.8 \times 10^{-157}$ ].

Because the associated SNPs in TOMM40 were located within the LD region containing APOE, TOMM40's strong association with AD was attributed to APOE; that is, the TOMM40 SNPs were correlated with either the e4, e3 or e2 epsilon allele SNPs of APOE. In reality, if the association of APOE4 to AD had not preceded these GWAS data by more than two decades, TOMM40 may have been identified as the AD gene instead of APOE [18,19].

Contrast this with the genetics of the amyloid precursor protein [APP] region on chromosome 21. Even though there are rare, autosomal dominant APP mutations, there is no GWAS support for the amyloid precursor protein [APP] region on chromosome 21 for sporadic, late-onset AD [20]. These rare mutations in APP that result in early onset, autosomal dominant AD have been used, in part, to justify the huge investment in amyloid research and

amyloid-associated mechanisms for drug development programs for late-onset AD [LOAD]. A pathogenetic mechanism involving these specific APP mutations has yet to be defined. However, independent of any APP mutation, it was observed in 2005 that APP protein truncated at the carboxyl terminus accumulates in the Tom40 mitochondrial import channel in the brains of AD patients and this interaction results in mitochondrial dysfunction [21<sup>\*\*</sup>,22<sup>\*</sup>].

Early positron emission tomography [PET] data from several centers demonstrated decreased glucose metabolism in brain regions associated with AD pathology in normal individuals carrying one or both APOE4 alleles compared to non-APOE4 carriers [23<sup>\*\*</sup>,24<sup>\*</sup>,25]. Since the brain depends on glucose, oxygen and the blood circulation needed to transport them, we investigated a hypothesis that there was something wrong with glucose energy efficiency and in 1996 began looking at drugs that increased the number of intracellular mitochondria as potential treatments [26<sup>\*\*</sup>].

In 1998, SNPs from the PEREC-1 gene next to APOE within the LD region were determined to be associated with risk for AD [27<sup>\*</sup>]. At that time a function for the PEREC-1 gene was unknown. In 2002, as a consequence of the Human Genome Project, PEREC-1 was discovered to be TOMM40 — the outer mitochondrial membrane channel through which peptides and proteins are imported into mitochondria to support mitochondrial function and biogenesis [28]. Because TOMM40 is located immediately adjacent to APOE on the genome, rather than simply assuming that the association between TOMM40 and AD just reflected linkage to the corresponding APOE alleles, both TOMM40 and APOE could be viewed as individually important in a mechanistic pathway to slowly damage mitochondrial dynamic

functions. This view of two genes that were in linkage disequilibrium and encoded biologically interacting proteins did not permeate the AD community even after phylogenetic analyses demonstrated APOE4, APOE3, and APOE2 alleles were linked to different alleles of a polyT locus, rs10524523 (henceforth, '523'), in intron 6 of TOMM40. In Caucasians these 523 alleles are defined as Short (S), Long (L), and Very Long (VL). Genotypes of the different polyT variant lengths at the 523 locus have been demonstrated to have different age of onset distributions. The APOE genotypes are fully represented using TOMM40-523 genotypes (Figure 3).

A group of researchers led by Dr. Yadong Huang at the Gladstone Institute [San Francisco, CA] investigated the dynamic functions of mitochondria in neuronal tissue cultures well before any AD GWAS data were published [21<sup>••</sup>]. They also investigated the role that intraneuronal apoE protein isoforms played in these dynamic functions,

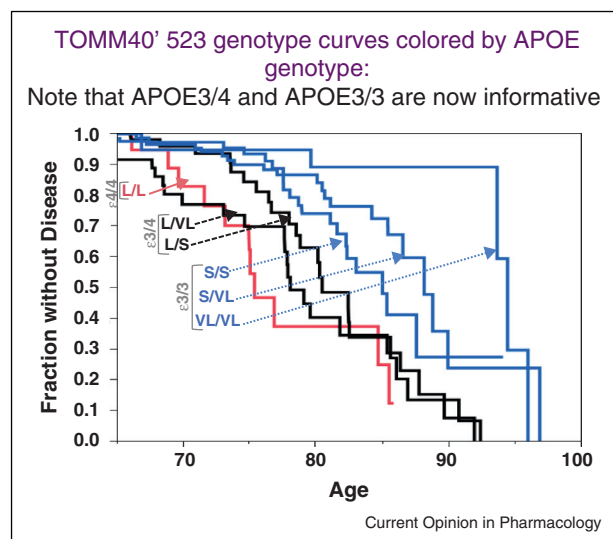
including the percentage of intraneuronal mitochondria moving within a neuron, how far and how fast they moved down the axon from the cell body to the terminal neurite area, the differential interaction of apoE4, apoE3, and apoE2 on these functions, and the effect of mitochondrial acting drugs in correcting dynamic functions and increasing neurite outgrowth [29<sup>••</sup>,30,31]. Earlier studies had also demonstrated the interactions of APOE, APP, and mitochondria [21<sup>••</sup>,22<sup>•</sup>,32].

In 2005, our research team at GlaxoSmithKline initiated phylogenetic mapping experiments looking directly at the LD regions on chromosome 19 that contains both APOE and TOMM40. In 2007, we reported that a 10Kb region that contains the TOMM40 gene produced a robust phylogenetic tree structure [33] and we later validated the phylogenetic tree structure in a different cohort and further demonstrated that there was a specific linkage between APOE and TOMM40 alleles (32). In subsequent experiments, it became clear that the TOMM40 region containing the intronic, variable polyT marker, rs10524523, contributed independently of APOE SNPs to the GWAS association results. Variations in the polyT lengths differentiated terminal clades on the phylogenetic tree and specific polyT lengths were differentially linked to downstream APOE genotypes. For example the APOE3 allele can be linked to one of two polyT alleles of rs10524523.

Phylogenetic mapping is a method that is commonly used to detect new evolutionary mutations in the viral genetics field. It has been used to map new mutation sites and the evolution of new strains of influenza viruses so that new vaccines can be manufactured annually. However, before the consensus human genome data were available, the methodology had not been applied to the analysis of human genetic complex disease loci. It is not a GWAS methodology, but rather a deep focus on a small region that has suspected importance. Critically, if a region of the genome shows low, to no, recombination, the approach is potentially very powerful for genotype/phenotype association analysis, based on the assumption that evolutionarily closely related sequences will share phenotypically important mutations.

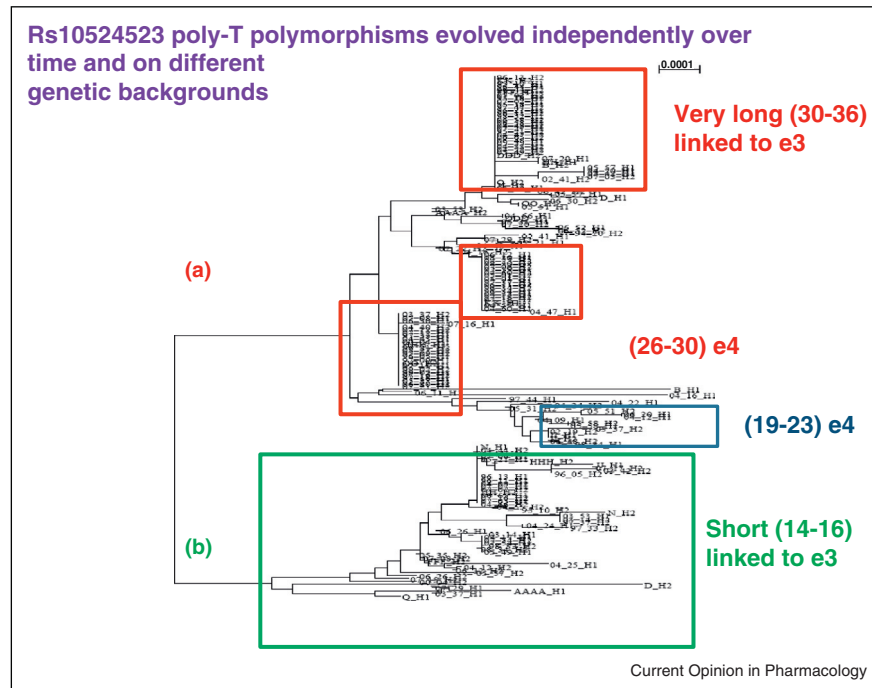
In 2009, the results of two independent phylogenetic mapping experiments of a small (10 Kb) region of the genome located within the larger LD region on chromosome 19 that contains both the APOE and TOMM40 genes were published [34<sup>••</sup>] (Figure 4). In an IRB-approved study of AD patients and age matched controls from a cohort from the Arizona Alzheimer's Disease Research Center, DNA was sequenced and underwent phylogenetic mapping. It was found that virtually all patients with the APOE4/4 genotype segregated into a specific branch, named Clade A. Clade B was almost completely (98%) homogeneous for the APOE3 allele.

**Figure 3**



Age at onset of cognitive impairment as a function of TOMM40-523 genotype. The Bryan Alzheimer's Disease Research Center (ADRC) Memory, Health, and Aging cohort ( $n = 508$ , 106 conversion events) was followed prospectively at the Bryan ADRC at Duke University. Cognitive status was determined using standard neuropsychological tests. The age at which cognitive impairment occurred was retrospectively stratified by TOMM40 genotype, and Kaplan-Meier curves were constructed. TOMM40 genotype was determined using a sequencing based genotyping method (Polymorphic DNA Technologies). TOMM40 genotypes and the corresponding APOE genotypes are indicated on the figure. The red line corresponds to APOE  $\epsilon$ 4/4; the two green lines correspond to APOE  $\epsilon$ 3/4, and the three blue lines correspond to APOE  $\epsilon$ 3/3. Note that within this cohort there were 78 individuals who carried an APOE  $\epsilon$ 2 allele, but only 5 developed cognitive impairment during the study (VL/L (APOE  $\epsilon$ 2/4),  $n = 1$ ; S/L (APOE  $\epsilon$ 2/4),  $n = 2$ ; VL/VL (APOE  $\epsilon$ 2/3),  $n = 2$ ). The number of subjects per 523 genotype (number converted to case status) was: 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). Reprinted from [31]. The risk algorithm for qualification in the TOMMORROW trial is illustrated in Ref. [31].

Figure 4



The sub-clades are differentiated by varying lengths of the poly-T allele (i.e. TOMM40-523). APOE3 can be linked to Very Long [Clade A, top red box] or Short 523 poly-T alleles [Clade B, green box] APOE4 is connected to Long poly-T alleles (Clade A, 2nd and 3rd red boxes,) and a small group of shorter Long polyT alleles [also Clade A, blue box]. Note especially that the APOE VL [Clade A] and the APOE3 Short [Clade B] clustered separately in distinct phylogenetic branches.

APOE3/3 subjects segregated to both Clades A and B, as did heterozygotes with the APOE3/4 and APOE2/3 genotypes. The phylogenetic tree was further distinguished by terminal clades, where clade membership was determined by the length of the polyT variant, rs10423523, of TOMM40. The boxes illustrated in Figure 4 show where polyT length variants from rs10524523 were distributed in clades by different size ranges. It was found that the Long [L] polyTs were generally on the same DNA strand as APOE4, while either a Short [S] or Very Long [VL] polyT strand was attached to APOE3 or the relatively uncommon APOE2. Most of the haplotypes containing L alleles of 523 were located in Clade A, but S or VL alleles of TOMM40 were attached to APOE3 or APOE2 strands and were divided to evolutionary-related branches between Clade A [VL] or Clade B [S]. Since S or VL variants of 523 were located on the same strands as APOE3 or APOE2, all other compound APOE genotypes containing APOE3 or APOE2 alleles were distributed between the major clades (Figure 3).

A prospective clinical onset study performed over two decades, with defined age of onset recorded, was examined retrospectively using the 523 genotypes. Kaplan–Meier analysis of 523 and APOE genotypes again showed that the APOE4/4 survival curve was identical to

523 L/L (Figure 3). The age of onset distribution curves from a large prospectively collected clinical population with accurate age of onset ascertainment demonstrated that the APOE4/4 curve reflected the 523 L/L carriers [35\*\*] (Figure 3). The APOE3/4 distribution now could be mapped as two age of onset distribution for 523 L-VL and L-S, and the APOE3/3 and APOE2 carriers were mapped to three curves, 523 S-S, S-VL or VL-VL. The 523 S or VL alleles attached to APOE2 are similar to APOE3, but APOE2 carriers shift the age of onset to 8–10 years older. (9) In this way, the phylogenetic mapping studies were pivotal in being able to elucidate the association between TOMM40 rs10524523 variants and risk of AD at a given age.

Several published studies attempted to simply look at AD onset data that were not determined from prospective, longitudinal studies with defined age of onset definitions, and thus lack the ability to differentiate genetic populations[36,37]. Figure 3 shows TOMM40 genotype-specific differences between the rapid decline in the proportion unaffected by dementia within a decade centered around 72–75 years of age [35\*\*].

Using TOMM40 genotype provides age-dependent risk prediction for everyone as opposed to the use of APOE

where only for the 2% of the Caucasian population that carry APOE 4/4 could an acceptable risk prediction be made. A biomarker risk algorithm, based on TOMM40 genotype, APOE carriage, and age at presentation has been developed that determines risk for clinical onset in 97% of the population. [Not enough subjects with the APOE2/4 or 2/2 genotypes, about 3% of the population, who developed AD were available from the prospective Caucasian study to produce the necessary age distribution curves] [35\*\*]. The TOMM40 algorithm derived from the age of onset distributions will be qualified for clinical applications during the TOMMORROW study. Using a validated testing methodology and prospective data, the clinical validation of the biomarker risk algorithm is one primary endpoint of the trial [38]. The trial tests a low dose of pioglitazone, a drug that has the potential to increase neuronal mitochondrial content [39\*\*], to determine whether it delays the onset of Mild Cognitive Impairment due to AD in cognitively normal subjects at high risk of developing disease within the period of the trial [35\*\*,40].

APOE and TOMM40 are in LD and can be clinically used as a prognostic device in Caucasians. There is great variability in the spectrum of 523 polyT lengths in other ethnicities, so studies of the relationship between TOMM40-523 genotypes and age of MCI of the AD type, or AD, onset are needed to generalize the risk algorithm to non-Caucasian ethnicities [5\*]. Further studies may demonstrate that each 523 genotype may be generalized and provide similar risk information across ethnicities, as is observed in other genetic diseases across ethnicities [5\*].

Disease onset prediction using these validated technologies can only be tested prospectively over time. It is important to note that the Phase III, TOMMORROW study, can also be the substrate for traditional efficacy PGX experiments to identify other specific, relevant genetic factors that contribute to the response or failure of response to pioglitazone for the treatment of MCI due to AD. Thus the efficacy PGX markers for treatment of MCI due to AD, or AD, in addition to the 523 genotype, may provide prognostic treatment information for already affected patients.

### **A very short follow-up on the use of HLA-B5701 as an informative test for risk of major allergic reactions: translation to medical qualification and the PGX-related data translating to a marketing success after patent expiry**

Qualification of HLA-B5701 was performed in a Phase III trial designed to prospectively qualify the marker for clinical practice. [1] What has not been stated in the literature is the creation of a commercial bonanza based on the discovery and qualification of B5701. As HIV

treatment regimes have progressed to use cocktails of drugs in combination preparations, the choice of abacavir to form the reverse-transcriptase function with preferred safety has led to continued use in formulations, and huge commercial earnings. The establishment of safety PGX led to a resurgence of abacavir sales *vis-a-vis* its newer competitors that still had no informative diagnostic for other side effects and adverse events. The subsequent use of abacavir in combination therapies has far exceeded the original commercial expectations. This is still the poster child for human PGX qualified by a prospective Phase III trial. The approximate cost of the trial was \$20 million, while the projected earnings are now extended well past chemical patent expiry.

In summary, clinical pharmacologic imaging and PGX uses are becoming intermixed in the vocabulary of drug development and commercial potential. The safety PGX that began in 1997, as a condition of accelerated market approval, revived abacavir use. The benefits of both the efficacy and safety of abacavir are still a source of commercial value — never anticipated during development — and are a victory for PGX by any measure.

The integration of new genetic technologies and PGX methods into the drug development enterprise is uncommon, except in cancer and infectious disease where efficacy can be clinically measured quite accurately and in shorter trials. Multiple genetic technologies were used to move forward with the TOMMORROW trial into uncharted drug development territory. Some very expensive popular technologies, like GWAS, may have been over-interpreted by the AD field. Hundreds of new genes have been nominated for disease associations by borderline statistical scores, yet to date phylogenetic mapping using quantitative clinical characteristics of disease has not been widely adopted, despite translational success. The TOMMORROW trial will qualify the genetic biomarker and test whether a known, safe drug given at low dose can be effective in delaying the onset of MCI due to AD in individuals at high genetic risk for their specific ages. Prospective delay of onset studies takes a long time to complete. In the interim, treatment studies for MCI-AD of shorter duration are being planned. Applications of phylogenetic analyses to other complex diseases of large medical need, such as obesity and schizophrenia, are currently underway.

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