

# The Medical and Economic Roles of Pipeline Pharmacogenetics: Alzheimer's Disease as a Model of Efficacy and HLA-B\*5701 as a Model of Safety

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Pharmacogenetics (PGX) is the study of drug response as a function of an individual's DNA. PGX is often viewed as an extension of disease association genetics, and although this information may be related, it is not the study of drug response. Although medicines are used to treat diseases, the value of strategies that identify and incorporate DNA biomarkers associated with clinical efficacy, or DNA biomarkers for untoward clinical responses, can be applied directly to pharmaceutical pipelines. The growth of adverse event PGX studies involving marketed medicines generally uses relatively large numbers of affected patients, but has been productive. However, the two critical strategies for pipeline genetics must make use of fewer patients: (1) the early identification of efficacy signals so that they can be applied early in development for targeted therapies and (2) identification of safety signals that can subsequently be validated prospectively during development using the least number of patients with adverse responses. Assumptions are often made that large numbers of patients are necessary to recognize PGX hypotheses and to validate DNA biomarkers. In some ways, pipeline pharmacogenetics may be viewed as the opposite of current genome-wide scanning designs. The goal is to obtain PGX signals in as few patients as possible, and then validate PGX hypotheses for specificity and sensitivity as development trials go forward — not using hundreds of thousand of markers to detect strong linkage disequilibrium signals in thousands of patients and their controls. Drug development takes 5–7 years for a drug candidate to traverse to registration — and this is similar to the timeframe for validating genetic biomarkers using sequential clinical trials. Two important examples are discussed, the association of APOE genotypes to the demonstration of actionable efficacy signals for the use of rosiglitazone for Alzheimer's disease; and the identification of HLA-B\*5701 as a highly sensitive and specific predictive marker for abacavir treated patients who will develop hypersensitivity syndrome (HSS). The rosiglitazone study prevented pipeline attrition by changing the interpretation of a critical Phase IIb proof of concept study (2005) from a failed study, to a positive efficacy response in a genetically predictable proportion of patients. Now, three years later, a Phase III program of clinical trials using pharmacogenetic designs is months away from completion (late08). If successfully registered (early09), millions of patients could benefit, and efficacy PGX would have achieved its first prospective block-buster. The use of safety candidate gene association genetics in patients who received abacavir therapy and developed HSS starting in 1998 culminated in a double blind clinical trial that determined sensitivity > 97% and specificity >99% in 2007. Clinical consensus panels rapidly recommended abacavir as the *preferred* therapy along with HLA-B\*5701 *pre-testing*, immediately increasing the market share of abacavir with respect to other reverse transcriptases that are associated with their own adverse events. Targeting of medicines during drug development is now possible, practical, and profitable.

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The drug industry definition of pharmacogenomics is 'the study of variations of DNA and RNA characteristics as related to drug response,' and that of 'pharmacogenetics' (PGX) is the study of DNA characteristics as related to drug response. Internationally harmonized definitions were recently published as a 'Guidance for Industry' document by the US Food and Drug Administration (E15 definitions for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories, 2008). PGX can be applied to two fundamental clinical questions

during drug development: (1) Are there predictors for drug candidate efficacy that can be validated in subsequent clinical trials? and (2) Are there predictors for side-effects, adverse events or metabolic differences that are associated with the use of the molecule in people? The first describes the potential enrichment of subpopulations of patients responsive to a drug; the second describes the risk of individuals for an adverse response.

PGX differs fundamentally from disease genetics based on the population at risk. For disease genetics, the population is open-ended, the PGX response only occurs in those individuals who receive the drug and not in anyone, patients or not, who has not been exposed to the drug (Roses, 2008c). It is, however, also possible to investigate disease genetics during clinical drug programs. PGX studies can determine associations related to the mechanism of disease clinical expression using patients enrolled in clinical trials to suggest additional targets for drug discovery. Disease mechanism genes have been frequently associated with PGX responses and therefore would also be included in candidate gene panels that are tested for PGX hypothesis generation. Candidate gene associations tailored to the knowledge or suspicion of a mechanism can also form either hypotheses for subsequent clinical trials, or provide a direction for specific target development. Thus disease associations and pathways can define PGX tools but also contribute to exploratory discovery.

During the 'pre-genomic' era before common genome-wide association screening [pre-2005], there were many instances where gene associations supported the involvement of disease mechanism candidate genes, or drug mechanism of action candidates. Hirschhorn *et al* (2002) reviewed reported gene associations with two or more replications and found that, although replication of reported associations was uncommon, when present it was frequently associated with genes related to the metabolism, binding or pathways. Goldstein *et al* (2003) similarly discussed the relevance of candidate genes related to the knowledge of the mechanism or pathway (Need *et al*, 2005; Roses, 1996, 2004).

'examples illustrate clearly defined groups of candidate genes that could harbor variation relevant to variable drug response: the first contains genes such as those that encode DMEs and drug transporters, which control the PHARMACOKINETIC properties (including DISPOSITION) of the drug; and the second group contains genes that encode drug targets (plus elements of the associated pathways) that influence drug PHARMACODYNAMICS. The second category includes not only the specific target of the drug, but also the broader pathway in which the target acts. To this we also add molecules that are similar to the target, which the drug might also modulate such as the effect that many non-cardiovascular drugs can have on potassium channels and thereby on QT-INTERVAL duration. One notable feature of pharmacogenetics is how often the obvious candidate genes carry variants that seem to influence drug response (Goldstein *et al*, 2003)'.

## GENOME-WIDE ASSOCIATION INTERPRETATIONS—THE 'LINKAGE' OF THE 21<sup>ST</sup> CENTURY

In the last two decades of the 20th century, genetic linkage studies proved very useful to map and identify the mutations associated with classical Mendelian diseases, including autosomal dominant, autosomal recessive, X-linked or mitochondrial genome disorders. These studies require the collection of family resources, including clinical data and DNA samples. Despite the difficulties of dealing with new forms of informed consents and clinical genetic counseling, particularly for normal family members at risk, linkage studies were incredibly successful in addressing genes for Mendelian-inherited diseases (McKusick, 2006, 2007). Of the thousands of clinical entities identified, most were extremely rare. Many common diseases were viewed as rather homogeneous and, in fact, prior to 1980 genetic etiologies were not strongly considered for cancers, Alzheimer's disease (AD), and many other common disorders (McKusick, 2006, 2007). The discovery of APOE4 as a major risk factor for 'sporadic' AD and the discussions of complex diseases that followed in the 1990s led to the rationale for supporting the Human Genome Projects and creating the tools for association studies and linkage disequilibrium (LD) mapping (Saunders and Roses, 1993; Strittmatter *et al*, 1993; Corder *et al*, 1993). Complex diseases have indeed been complex, and the failure to replicate the enormously statistical significance for APOE4 was rationalized by assertions of great genetic heterogeneity. However, failure to rapidly find many gene associations in complex diseases does not prove that they cannot be found by the application of other technologies. At this point in time, genome-wide association studies are identifying large numbers of genes with 'almost' statistically significant results (McCarthy *et al*, 2008). What are missing are newer methods to determine the effects of two or more associations: how to combine biology with genetics. A forerunner for this type of experimental strategy is provided in a short discussion of a second major interacting gene for AD, summarized later in this communication. The use of densely genotyped candidate pathway genes reduces the number of statistical tests, provides specificity with limited number of patients, and is being "piloted" in pipeline safety PGX, and may soon be able to decipher the complex interactions of complex diseases (Martin *et al*, 2000; Zaks *et al*, 2006; Ahmadi *et al*, 2005; Roses, 1997).

The current limited experience with genome-wide SNP scanning using 500,000 or more than a million SNPs has created a fascinating situation. (McCarthy *et al*, 2008; Frayling, 2007; Zanke *et al*, 2007; Warren *et al*, 2007; Zeggini *et al*, 2007; Sladek *et al*, 2007; North *et al*, 2004; Libioulle *et al*, 2007; Wellcome Trust Case-Control Consortium, 2007; Raelson *et al*, 2007; Coon *et al*, 2007; Scott *et al*, 2007; Hunter *et al*, 2007; Schymick *et al*, 2007; Li *et al*, 2008). The time required to genotype and analyze the data derived from pre-existing clinical collections can be as little as

several weeks. However, because of the large number of SNPs that need to be considered, the statistical corrections for most of these studies in complex diseases have resulted in only a few associated genes with more than borderline statistical significance. A greater number have borderline significance, which surely includes false-positive gene loci. Some may confirm independently previously performed genetic linkage and association studies (see Diabetes Mellitus below), but others may be expected to be false-positives (Ioannidis, 2007).

Large well-characterized collections of several thousand patients and controls are now recognized as limiting factors, specifically assessing and accessing clinical phenotypes, the time and expense to identify and consent patients and controls, and the lack of availability of readily available populations for confirmation. The amount of work to collect, consent, examine and test thousands of patients is enormous and expensive—yet as data from additional diseases become available several unfortunate realities are becoming clear. The first is that most complex diseases, unlike the AD example, do not have extremely highly significant associations ( $p \ll E-8$ ) after correction for the number of SNP tests ( $5E+5$ , or  $6E$ ) (Coon *et al*, 2007; Li *et al*, 2008). The second reality is that there are usually 10–100 genes identified with borderline significance suggesting that many large clinical series will need to be combined (Wellcome Trust Case Control Consortium, 2007). The third reality can be disappointing to many scientists with relatively fixed hypotheses—many widely accepted popular candidate genes are located within LD groups of genes that demonstrate no significant genome-wide association signal using genome-wide association scanning (Roses *et al*, 2007b; Meng *et al*, 2007; Dreses-Werringloer *et al*, 2008; Anandatheerthavarada *et al*, 2003).

In AD research, the amyloid precursor protein (APP) (metabolism and consequences) has been at the center of modern AD research, even earlier than the 1988 discovery of the APP gene and the subsequent genetic mutations associated with a rare early-onset form of the disease (Strittmatter *et al*, 1993; Schmechel *et al*, 1988; Bahmanyar *et al*, 1987; Goldgaber *et al*, 1987; Goate *et al*, 1991; St George-Hyslop *et al*, 1987; Tanzi *et al*, 1987; Citron *et al*, 1997; Sherrington *et al*, 1996; Kamino *et al*, 1996; Schellenberg *et al*, 1992). Current therapeutic agents related to amyloid are being investigated in early clinical trials but, to date, clinical benefit to patients has been elusive (Okura and Matsumoto, 2008; Pride *et al*, 2008). However, although the APOE gene is located within an LD region associated with an extraordinarily high significance, the APP gene does not appear to be associated with AD using genome-wide association scanning (Roses *et al*, 2007b). The so-called ‘Amyloid Cascade’ involving caspase enzymes may actually be a downstream consequence of mitochondrial toxicity (Townsend *et al*, 2007; Selkoe and Wolfe, 2007; Walsh and Selkoe, 2007; Dunys *et al*, 2007; Lee *et al*, 2007). APOE4 and APOE3 fragments without the C-terminal region may well be a form of pro-apoptotic death signals, often discussed in

the oncology literature, with APP one of many catabolized molecules—but one which forms aggregates in tissue (Ziedan *et al*, 2008; Ashkenazi and Herbst, 2008; Hisatomi *et al*, 2008; Mahley *et al*, 2006; Roses *et al*, 2007a).

Unfortunately, there appears to be a relaxation of the rules of evidence for genes identified by genome-wide association (Dahlman *et al*, 2002). Formerly, because of the lack of validation observed with most reported association, when candidate gene papers were reviewed for publication in high quality journals, replication became a frequent requirement and biological validation studies were also included in the publication. Over several decades it had become clear from the literature that less than 5% of published disease association journal reports were ever confirmed and validated (Hirschhorn *et al*, 2002; Dahlman *et al*, 2002). McCarthy *et al* (2008) recently reported that more than 50 disease genes were found to be associated with a variety of diseases using genome-wide association scanning. This report “identified genes” which were not yet replicated, even between each of several genome-wide association studies of the same disease (see below). Very few met the candidate criteria adopted before genome-wide association scanning.

#### EXAMPLE: TYPE 2 DIABETES MELLITUS (T2DM)

The data from multiple T2DM genetic studies support the view that very large clinical case–control series are necessary to provide statistical significance for loci ‘identified’ by genome-wide association scanning. Increasing the density of coverage to over a million SNPs may, in fact, make it more difficult to identify statistically significant loci by needing to introduce a larger statistical correction. As an example, a published T2DM genome-wide association scanning was performed as part of the Wellcome Trust Collaborative Group study of seven diseases (Wellcome Trust Case Control Consortium, 2007). The analyses revealed three loci of significance—each barely below the corrected significance levels and nothing as dramatic as the APOE region of AD (Wellcome Trust Case Control Consortium, 2007). The association of the transcription factor 7-like gene (TCF7L2) illustrates the interplay between genome-wide association scanning and the previously more focused genetic association and linkage studies. In this case, genome-wide association scanning actually confirmed earlier gene linkage and association data. After reporting linkage of T2DM to chromosome 10q, Grant *et al* (2006) genotyped 228 microsatellite markers to Icelandic individuals with T2DM and controls over a 10.5-Mb interval on 10q and reported linkage. Although it is clear that most reported linkage and targeted association results can result in many false-positives, these investigators found that a microsatellite variant within intron 3 of TCF7L2 was associated with T2DM, ( $p = 2.1E-9$ ) (Hirschhorn *et al*, 2002; Helgason *et al*, 2007). This was then replicated in a US cohort, ( $p = 3.3E-9$ ). These data were then confirmed by

genome-wide association scanning containing ~500 k SNPs in 2000 patients (Wellcome Trust Case Control Consortium, 2007). In this case the gene linkage and gene association studies were performed prior to genome-wide association scanning, and *TCF7L2* was confirmed ( $p = 5.7E-13$ ). Two other previously reported loci, *FTO* (chromosome 16) and *CDKAL1* (chromosome 6) were also suggested by genome-wide association scanning, but with more modest  $p$ -values.

Sladek *et al* (2007) also ‘identified’ four other loci containing variants that confer risk for T2DM, in addition to confirming the association with the *TCF7L2* gene. These loci include “a non-synonymous polymorphism in the zinc transporter *SLC30A8*, which is expressed exclusively in insulin-producing  $\beta$ -cells, and two linkage disequilibrium blocks that contain genes potentially involved in  $\beta$ -cell development or function (*IDE-KIF11-HHEX* and *EXT2-ALX4*.” (Sladek *et al*, 2007) Other than the *TCF7L2* confirmation, the other three loci (on chromosomes 8, 10, and 11) were different than the second and third loci (chromosomes 6 and 16) in the Wellcome Trust study (Wellcome Trust Case Control Consortium, 2007). Follow-up dense mapping of these particular regions may find additional associated SNPs and add support to a locus of borderline significance (Zaks *et al*, 2006; Roses, 2008a). This example, however, illustrates the interplay between genome-wide association scanning and more focused association studies. It may actually be more difficult than investigators imagined discovering new genes using primary genome-wide association scanning data. After genome-wide association scanning, more focused secondary candidate analyses are needed in independently ascertained sets of patients to confirm borderline significant SNPs or regions.

## EXAMPLE: WHERE DOES AD DISEASE GENETICS END AND PGX BEGIN?

Genome-wide association scanning data from studies of AD patients have unequivocally identified the LD region that contain the apolipoprotein E gene (Coon *et al*, 2007; Li *et al*, 2008). One variant, *APOE4*, has been widely replicated as a confirmed susceptibility gene since the initial publications in 1993 (Saunders and Roses, 1993; Strittmatter *et al*, 1993; Corder *et al*, 1993). However, the genome-wide association scanning data resulted in a remarkable ‘coincidence’ observed in cell biology studies involving the co-localization of *APOE* and *tomm40* proteins to the outer mitochondrial membrane. This interacting gene, *TOMM40* (the outer mitochondrial membrane channel gene) was first encountered during 1997 genetic epidemiology studies modeling whether LD mapping around *APOE* would have found the gene faster than the years it took 5 years earlier in 1992 (Lai *et al*, 1998). The then-unknown genetic polymorphisms, called *PEREC*, were located adjacent to *APOE* within a small LD region. *APOE* co-localizes to the outer mitochondrial membrane, suggesting isoform-specific interactions leading to a potential role for *APOE*-induced mitochondrial apoptosis as an early step in AD expression (Chang *et al*,

2005). Biological data have demonstrated that the proportion of mobile mitochondria in neuronal cell culture, as well as the speed at which they move and the distance that they traverse, lead to increased mitochondrial apoptosis (Mahley *et al*, 2006; Roses *et al*, 2007a; Xu *et al*, 2006; Harris *et al*, 2003; Brodbeck *et al*, 2008). Phylogenetic data suggesting an independent genetic effect on the development of AD for *TOMM40* are outlined below. The cis-acting event of a then-unknown protein in 1998 produced the Alzheimer-like pathology in *APOE* knockout mice that were transfected with human genomic DNA cosmid fragments (Roses *et al*, 1998). This was before the identification of *TOMM40*, or its adjacent location within an LD region.

The interaction between multiple haplotypes involving *TOMM40* variants and *APOE* isoforms highlight an independent contribution of *APOE3* to AD pathogenesis (Roses, 2008a, c; Li *et al*, 2008). Several of the *TOMM40* gene variants evolved only cis-linked on *APOE3* cistrons. Thus any added genetic effect of the *TOMM40* variants segregates independently from *APOE4* cistrons. This ‘coincidence’ of adjacent interacting proteins may account for the extraordinarily significant statistical association data found in all AD genome-wide association scanning studies. It is of interest to note that the initial commercially available genome-wide association scanning platforms did not contain any *APOE* polymorphisms but were identified with *TOMM40* and *APOC1* SNPs—but the region is virtually always referred to as the ‘*APOE*’ region. Perhaps the *APOE* and *TOMM40* region would be more accurate with respect to AD pathogenesis.

These data, which combine disease genetics and putative molecular mechanisms of pathogenesis, can also be viewed within a clinical PGX context. Because of the strong genetic effect of inheriting an *APOE4* allele, *APOE4* has been referred to as a complex susceptibility gene for almost two decades. Consistent replications of the age of onset distributions as a function of *APOE* genotype confirm that the role of *APOE3* inheritance is not totally benign, but is a lower risk factor observed at a slower rate of disease onset (Corder *et al*, 1993; Li *et al*, 2008). There are genetic variants of *TOMM40* that are located only on DNA strands containing *APOE3* in the LD regions (Roses *et al*, unpublished data), and thus not in Hardy–Weinberg’s equilibrium as was required for SNPs qualifying for inclusion on genome-wide association panels. Phylogenetic changes in *TOMM40* sequences that are cis-linked only to *APOE3* can act to increase the risk of AD when they are on the same cistron as *APOE3*, whereas other variants of *TOMM40* cis-linked to *APOE3* decrease the risk associated with *APOE3*. An independent genetic test is to determine whether those *TOMM40* polymorphisms associated with less risk of AD segregate at a later age in onset of disease distribution plots for AD patients with *APOE3*-containing genotypes (*APOE3-3* or *APOE4-3*), (Roses, unpublished data).

From the rosiglitazone clinical trial program detailed below, it has been possible to collect *APOE* allele-specific patient groups (*APOE4*-negative and *APOE4*-positive) to

further confirm published age of onset data in a set of patients specifically encountered in a clinical trial setting. From the collections of DNA from treated patients in the trials, as well as patients not treated with rosiglitazone, it will be possible to independently treat the putative predictions emerging from the study of TOMM40 variants linked to APOE3 and not APOE4 or APOE2. Thus there is interplay between statistical genetics and mechanisms of disease—as well as drug mechanisms—that may be more clearly illustrated following the conclusion and analyses of the rosiglitazone AD clinical trials in 2009.

### EXAMPLE: THE HISTORY OF THE MECHANICS OF AN AD CLINICAL TRIAL WITH AN EFFICACY PGX PROSPECTIVE HYPOTHESIS

The most important point along the drug discovery and development pipeline is the demonstration of proof of concept for efficacy (POC). There are really two factors that determine whether a drug candidate is deemed effective. If the POC is prospectively predicted in the protocol for a clinical trial, then the regulators consider the trial as hypothesis testing. But if the results (clinical or PGX) are determined *post hoc*, then the data are considered exploratory and must be tested prospectively in subsequent trials. The difference between two sequential positive hypothesis testing trials and three longitudinal conformational registration trials can extend the development program by several years. Clinical trials frequently take many months or several years to set up and complete. The importance of testing prospective hypotheses in the earliest stages of drug development (especially in terms of the always-expiring market exclusivity) is a major commercial factor. Even if 200 prospective hypotheses are simultaneously tested in the initial Phase IIA trial, and only one seems to predict efficacy, then the developer may be one step closer to confirmation and possible drug registration (Roses *et al*, 2007a).

Rosiglitazone for the treatment of AD provides a clear demonstration of a failed study in the intention-to-treat (ITT) patients. Like many such studies, this program would have been terminated and efficiently dropped from the portfolio in less than a week after results became available. However, because there was a prospectively defined hypothesis—in this case, that APOE4 carrying AD patients would respond less well than AD patients who carried no APOE4 alleles, efficacy for APOE4 non-carriers was defined within a week following the original ITT analyses. It is important to understand the timing for pipeline PGX. Clinical data become available only after the conclusion of trial and the phenotypic data define the efficacy analyses of the genetic hypotheses. Efficacy PGX analyses can only commence following the identification of those patients who respond with clinical end points of efficacy. Even in this case, it took an intervention by the Chair of R&D to

hold back the efficient killing of a program even for 1–2 weeks to complete the PGX analyses. Attrition can be habit forming and insanely efficient.

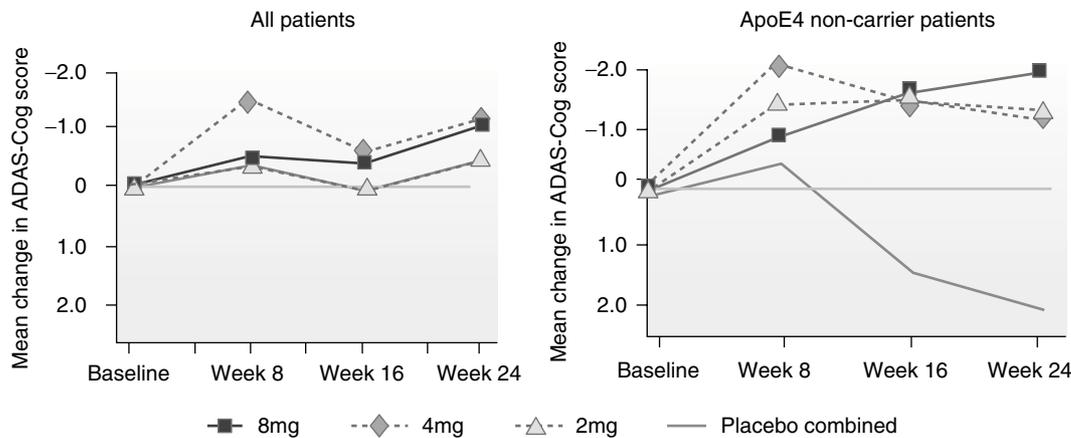
In this case, within a week after the patients who responded with efficacy were identified, a totally ‘failed’ experiment was converted to a successful efficacy PGX result for which the clinically responding population had been predicted by the prospective hypothesis. This was a textbook case of preventing attrition, and the effect that efficacy PGX can have on the critical POC step in drug development. In AD, the clinical end points for efficacy accepted by the regulators had been met by approximately half of the ITT patients—but a predictable half of a huge population with a severe, unmet medical need.

Phase III involved recruiting and following thousands of patients for 48-week clinical trials. These trials, and their PGX designs, must be pre-planned to be efficiently initiated and mobilized. In the most efficient circumstances of pipeline pre-planning, working with the regulators with respect to the End of Phase II meeting, establishing the new clinical centers, and recruiting patients can take a year or more to get off the ground. The clinical trial actually starts the day that the first patient enters the study and ends the day that the last patient completes the study. The first two studies began in 2005 and will close before the end of 2008. The jury is still out (currently August 2008) but a verdict is expected in early 2009.

Figure 1 illustrates the Phase II data (Risner *et al*, 2006). On the left side are the results of treatment with three different doses of rosiglitazone monotherapy over a 24-week study. Under any conditions of interpretation, there is absolutely no evidence that there was any efficacy in any treated group of patients in this study, nor in the placebo group—a dismal failure. These were the initial data available at the end of the study.

However, after PGX analyses and a week delayed, there was genotype-related efficacy for only those patients who did not carry an APOE4 allele (approximately 50%). Each of the groups receiving one of the three doses illustrated a measurable improvement on the Alzheimer’s disease Assessment Scale for cognition (ADAS-cog scale, accepted as a primary end point by the regulatory authorities), as well as secondary end points. The combined placebo group at all three doses for all APOE4-negative AD patients did not improve but demonstrated slight deterioration over the 24 weeks of the Phase IIB monotherapy study. The difference between the placebo and the drug met the ‘three point criteria’ expected by the FDA for the ITT group who carried no APOE4 allele. Of course, as would be predicted from the whole ITT population data, carriers of one or two APOE4 alleles showed no improvement.

An important new process in the FDA, the Voluntary Genomic Data Submission, allowed a non-decisional discussion with the FDA leadership regarding the interpretation of the PGX data, and proposals for the Phase III registration programs (Orr *et al*, 2007; Lesko, 2007). A spirited VGDS discussion allowed a transparent debate to



**Figure 1.** The left panel represents the ADAS-cog results for all patients who were treated with rosiglitazone with any of the three doses versus their controls. Negative values are associated with clinical improvement, with an effect of approximately  $-3$ U when compared with placebo-treated patients is considered actionable for consideration for a phase III registration program. There was no evidence of efficacy. The right panel illustrates the effect of three different doses of rosiglitazone for all treated patients who did not carry an APOE4 allele [APOE3/3 or APOE2/2 genotypes]. The prospective hypothesis in the study protocol was that APOE4 carriers would not respond to rosiglitazone as well when compared to non-carriers. [see text] The combined patients who received placebo are also illustrated, and a positive clinical improvement is observed. Thus, the PGX hypothesis appeared to be confirmed in a proof-of-concept phase IIB study.

occur—most of which involved the views of various experts within the FDA. A consensus view of the possible designs of a Phase III program allowed the End of Phase II Meeting (held the following month) to subsequently proceed efficiently. An APOE-specific clinical trial design, which would evaluate APOE4-negative and APOE4-positive patients was adopted. By recruiting patients with each of the four common genotypes, APOE3/3, APOE4/4, APOE3/4 and APOE2/3 (only a few APOE2/4 and APOE2/2 individuals will be recruited) genotype-specific analyses will be available. Two different doses of rosiglitazone were used with the low dose equivalent to 25% of the commonly used daily dose for the treatment of T2DM. Of particular interest, besides efficacy, in these large studies is whether there will be any genotype-specific side-effects or adverse events (AEs). One important fact that is certainly diagnosable before the study concludes is that there is no increased frequency of myocardial infarction deaths in any of the three phase III populations to date. This issue was raised inappropriately for T2DM treatment based on a flawed statistical meta-analysis assuming all cells of data were equivalent and using a US Congressional government hearing to damage the reputation of rosiglitazone, resulting in a delay of recruitment late in the course of the AD trials (Nissen and Wolski, 2007; Goldberg, 2007; Fovenyi, 2006; Gerstein *et al*, 2004, 2006; Lago *et al*, 2007).

## TWO MORE RECENT CLINICAL TRIALS REPORT IMPROVEMENT IN APOE4 NON-CARRIERS: ANOTHER COINCIDENCE?

There have been two press reports involving two quite different therapeutic agents that associate an efficacy

response in treated AD patients to the presence or absence of an APOE4 allele. Each is leading to a PGX design that will be used in additional clinical trials. If these proposed treatments are approved in subsequent years, then the possibilities for multiple-drug therapies affecting pharmacogenetic genetic subgroups of patients may become a reality. In fact, if rosiglitazone is approved in 2009, it may become incumbent upon follow-on drug development programs to demonstrate non-inferiority, or even complementary or additive effects.

Accera, a small biotechnology company, announced its plan “to commercialize a first-in-class medical food product to treat Alzheimer’s disease, called Ketasyn, which is particularly effective in APOE4-negative patients.” ([http://www.pgxreporter.com/issues/5\\_43/features/143237-1.html](http://www.pgxreporter.com/issues/5_43/features/143237-1.html)).

Similarly, recently announced results from Phase II clinical trials for the investigational Alzheimer’s drug bapineuzumab (an antibody to clear amyloid  $\beta$ -peptide) shows the drug may be beneficial in patients who are non-carriers of the APOE4 allele. It was also mentioned that there may be a higher incidence of vaginal edema in APOE4-positive patients. ([http://www.pgxreporter.com/issues/6\\_27/features/147947-1.html](http://www.pgxreporter.com/issues/6_27/features/147947-1.html)). Whether these initial reports are validated, the fact remains that allele-specific APOE subsets of patients have been defined by apparent clinical responses—that will be tested in subsequent clinical trials.

From a PGX point of view, it is interesting to note that both developers of other early AD treatment programs performed *post hoc*, retrospective PGX evaluations of their data using the inheritance of an APOE4 allele, and have reported targeted ‘successes.’ Both of these drug candidates have yet to enter Phase III trials, and further data supporting the press reports are not yet available. However, because neither company had proposed the PGX hypothesis

prospectively, each study can only be considered as ‘hypothesis generating,’ nor could either trial be used as one of the two positive studies required for drug registration, that is, two more studies would be required at a minimum. In this case, Ketasyn will probably be tested as a medical food.

## A COMMENT ON THE TENSION BETWEEN AN R&D VIEW AND A COMMERCIAL VIEW

If rosiglitazone is approved and reimbursed in 2009, the commercial view of efficacy PGX will change rapidly—many years after the R&D strategy was conceived and executed. The same industry analysts who discount the chance of success in 2008 may be the ‘earliest’ proponents of pipeline PGX in 2009. Nothing will have changed in PGX science—only the perception of those who control the R&D budgets within the pharmaceutical industry. PGX predictive science is evaluated prospectively, using experimental strategies that took many years to pilot. Economic success is always evaluated by the ‘here and now’.

## OTHER NEUROPSYCHIATRIC AND COMPLEX CLINICAL APPLICATIONS OF PIPELINE PGX

In the context of neuropsychopharmacology there are some special difficulties in evaluating clinical trials. Using the historic paradigm of determining the difference of an average response in defined clinical end points with the active drug candidate or placebo, it has been extremely difficult to generate consistent efficacy with many newer agents (Malhotra *et al*, 2004). This raises the question of whether the efficacy responses observed with some of the older drugs are appropriate for different mechanisms of action of newer drug candidates. Most clinical trial designs were based on historic assumptions regarding the homogeneity of neurological and psychiatric diseases. This may have contributed to defining end points for studies that only powerful drugs could meet, but unfortunately are also associated with very significant side-effects and adverse events (AEs). On the other hand, experience with several decades of psychiatric treatment has created a knowledge base for PGX-based candidate gene panels derived from those mechanisms.

Serretti *et al* (2008) have recently reviewed PGX studies in depression and proposed methodological guidelines not only including end points but also sampling, diagnostic criteria, compliance and trial designs. There have been many PGX studies in mood disorders “after the initial reports linking gene variants to treatment outcomes. However, a considerable range of methodologies has been used, making it difficult to compare results across studies.... Specification of sampling source (inpatients vs outpatients, primary vs tertiary settings), standardization of diagnostic systems and treatments, adequate monitoring of compliance through

plasma levels, sufficient length of observation (at least 6 weeks for acute antidepressant treatments, though 3–6 months are preferable), the use of a range of response criteria and the inclusion of possible environmental confounding variables (life events, social support, temperament) are all potentially important issues when planning pharmacogenetic studies.” This scholarly review presents suggestions and recommendations for approaching confirmation of depression studies and other mood disorders.

Oncology and virology are therapeutic areas that have led the way in determining the PGX responses to drugs, in no small part aided by the knowledge of basic mechanisms and the ability to get drugs into the marketplace faster because of accelerated marketing programs in the United States, and conditional approvals in the EU (Anonymous, 1992). It is interesting to contrast this situation with the extreme heterogeneity of inherited psychiatric and neurological diseases. DNA variants in many different genes in the genome frequently define virtually identical phenotypes in disease categories that had previously been considered under one clinical diagnosis, such as the hereditary spinocerebellar diseases, hereditary spastic paraparesis and others (Jani-Acsadi *et al*, 2008; Russman, 2007; Koeppen, 2005; Passamonti *et al*, 2004). There are now tens of specific and diverse mutations, which define various endo-phenotypes and inheritance patterns. It is unclear whether, even in these genetically classified disease groups, similar treatment will be successful. Therefore, why should we assume that complex neuropsychiatric diseases are different simply because we still have difficulty in identifying the contributing genes, and measure an ‘average response’ to drug therapy across heterogeneous patients vs placebo? Perhaps this is a clue that complex diseases like schizophrenia and depression are ‘complex’ and heterogeneous perhaps not.

Diseases have often been defined by drug-responsiveness before PGX entered the lexicon. Steroid-responsive asthma was an example of category of description over many decades without knowledge of mutations, polymorphisms, or the basis of steroid-responsive heterogeneity. Depression, schizophrenia, Alzheimer’s dementia, obesity, heart diseases, and other complex neuropsychiatric ‘diseases’ are frequently subclassified by pathology or drug-responsiveness—with little idea whether there are many etiologies and certainly no methods of prediction. One might assume that these ‘diseases’ may well become drug-defined when efficacy PGX allows a better prediction of responsive phenotypes. To better understand the role of PGX in drug development, pharmacovigilance and future medical care, some recent examples will be presented and briefly discussed.

## SAFETY PGX—FOR THE DEVELOPERS IT IS ALL ABOUT TIMING

The list of clinical trials in depression that have had one successful trial and multiple efficacy failures is long.

The trials are dependent on all the factors mentioned above, as well as two over-arching facts: (1) the clinical definitions used in the studies, even if internally consistent, may still result in the collection of a different spectrum of clinical heterogeneity in subsequent studies, even with the same drug and the same physicians; and (2) failure to reproduce the successful trial design using PGX data. Similar to these data presented above for APOE alleles and AD, it may be possible to define more accurate and responsive patient selection using genetic markers and PGX trial designs (Roses, 2008b).

Adverse events are not specifically related to only neuropsychiatric drugs, but represent a common problem for all drugs. When the end points of a trial are defined clinically, as in many neuropsychiatric studies, a stronger drug is generally needed to significantly affect end points during the timeframe of the trial. Small subtle effects are difficult to quantify and may take longer to be appreciated by current clinical end point measurements. Stronger drugs and higher doses amplify the appearance of adverse events.

From the drug developer's point of view, the management of adverse events is all about timing. When uncommon, but not rare, side-effects or adverse events are noted during development, efforts should be made to understand any genetic contribution to the susceptibility for experiencing the adverse event—not trying to ignore its existence or using speculative rationales to put it aside. The difference is the ability to develop diagnostic biomarkers using PGX strategies. The clearest example in the literature is abacavir, a non nucleoside reverse transcriptase that is used to treat HIV infection. Approximately 5% of patients who started the drug can experience a hypersensitivity syndrome (HSS) consisting of fever, rash, gastrointestinal symptoms and constitutional symptoms. The onset of most HSS occurs within 6 weeks of initiating abacavir in naive patients and can be managed by stopping abacavir therapy for alternative drugs now in the marketplace. Re-treatment of HSS patients with abacavir can lead to anaphylactic shock and death (Hetherington *et al*, 2001).

In 1997 abacavir was the first of its class—for what was then an almost invariably fatal disease. The regulators approved an accelerated marketing program for treatments of cancer and HIV, which allowed potentially life-saving drugs to be available to patients, but under a strict set of enforced risk management procedures. Included in the accelerated approval for abacavir was the contract to develop a predictive diagnostic marker to avoid dosing susceptible patients. A detailed historic review of the chronology of a diagnostic test for abacavir-induced HSS was recently published (Hughes *et al*, 2008).

From a public health point of view, acknowledging that all drugs have side-effects, accelerated approval allows patients to receive the benefit early in development and essentially mandated a risk management program that included active surveillance by the sponsor—not just voluntary reporting to a central database. The advantage to the patients is that the significant part of the revenue of the marketed drug

must be devoted to enhancing safety. With regard to the industry, when adverse events and serious side-effects arise post-marketing for some drugs, the regulators can only use blunt tools for enforcement: either black-box warnings added to the drug label, or withdrawal of the drug from the marketplace. Over the past few years some very high profile drugs have been withdrawn from marketing and suffered serious legal and financial penalties—besides having already spent the entire costs of development, launch and marketing (Peck, 2007). The economics of safety defines the solution—learn what you can as early as possible and develop risk management plans that can define diagnostic tests.

Well before the genome was declared to be sequenced, two groups acting independently reported retrospective studies of HSS cases demonstrated a highly significant percentage of the HSS patients carried HLA-B\*5701 (Hetherington *et al*, 2002; Mallal *et al*, 2002). GSK sponsored a large prospective clinical trial design to measure the value of pre-screening HIV patients with HLA-B\*5701 and the frequency of HSS. In patch tested immunologically confirmed patients, this result was dramatic with both the specificity and the sensitivity of the test exceeding 97%. None of the screened group developed HSS, whereas approximately 3% of the non-screened group who tested positive for the marker developed HSS (Mallal, 2007, 2008).

The economic effect is real. Abacavir is now the preferred treatment for initiation of therapy in naive patients, with the recommendation that all patients should be screened before initiation of treatment (Phillips and Mallal, 2008; Adult and adolescent guidelines HIV [abacavir], 2008). The economic consequences for the company (and the benefits to patients) reflect the fact that the drug has enormously increased its benefit to risk ratio. From a company perspective, the increase in revenue over previous projections in 1 year paid for the entire risk management program many times over. It is the first clear example based on the gold standard—a prospective double-blinded clinical trial of an adverse event, but it is certainly not a special case, and will be repeated many times over as regulators require and enforce better surveillance and risk management programs in the future (Mallal, 2008).

Everyone wins with safety. Not being proactive about safety by using existing PGX tools can only put a drug at risk. In fact, most drugs are not approved as accelerated marketing but become available to physician use only after launch. This creates a nightmare situation for drug companies—every academic investigator with access to treated patients can rapidly investigate safety signals—whose only effect for the pharmaceutical company is negative. Why not protect the drug proactively and relatively inexpensively? Patients are safer, payers are more likely to reimburse, regulators are satisfied, and the asset is in an improved position in the marketplace. For companies with a reputation for embracing safety, the added gains of increased stock ownership and value will also be a factor defining success.

## PGX AS A BUSINESS DRIVER

The most important factor in drug discovery and development is reducing attrition, or failure once an asset has had a positive POC and is in the expensive phases of full development. The extreme negative case would be a fully developed molecule with additional Phase IV programs, which is removed from the market because of severe ‘post-marketing’ adverse events after total investment had been committed (Maron and Hauser, 2007; Epstein, 2007). In an industry where successes have been defined as a drug with a \$1 billion per year market, a few successes can sustain an otherwise productive company. However, included in the current huge estimates for cost of drug development are the costs of each failure (Reichert, 2003). Over the past decade estimates of drug costs have continued to increase because of the continuing attrition. When research and development are measured as a necessary expense, rather than the lifeline of a pharmaceutical company, the short-term economic perspective can impede productivity. Although the literature is full of high profile drugs that failed because of safety concerns, the pharmaceutical industry has been slow to protect its pipelines by the identification of those individuals at the greatest risk of adverse effects—and to use that information to increase the benefit to risk ratio. The mantra that a safety test would cut into sales is hollow, especially if the result is no sales (accompanied by expensive litigation) because of safety issues in full development or in post-marketing pharmacovigilance. Regulatory emphasis on evidence-based surveillance systems will increase the role of PGX to discover markers that predict the occurrence of an AE or side-effect that decreases the attractiveness of a safe and effective medicine. Yet the pharmaceutical industry continues to repeat failing procedures and to re-organize 10 to 12-year pipeline processes every 5–7 years. Albert Einstein, who was a reasonably perceptive individual said: ‘Insanity is doing the same thing over and over again and expecting different results’ ([www.brainyquote.com/quotes/quotes/a/alberteins133991.html](http://www.brainyquote.com/quotes/quotes/a/alberteins133991.html)). Yet increasingly obsolete perceptions within the financial and pharmaceutical communities ignore the chance to target medicines for safety and efficacy with existing PGX technologies.

Safety concerns are the main purview of regulators. Once some degree of efficacy is demonstrated, targeting patients for efficacy is actually a major concern for payers, both private and public. Reimbursement of an approved drug is the true measure of success. Registration of a drug with a ‘we can sell it’ strategy is becoming harder to sustain. The regulators defining safety more rigorously, but the payers control the marketplace. Targeting efficacy and safety through large PGX studies in health maintenance organizations or in publicly supported health systems will become far more important in the USA over the next decade—just as it already has become in European, Asian, and Canadian systems over the past decade. For pharmaceutical companies, the time to learn the most about your drug is during development and surveillance. Presenting a medicine that

has a greater than 90% chance of a successful therapeutic response in a smaller targeted population is of far greater value than trying to continue to target all patients—because the target value is obtainable and not a cherished relic of the past. The best reason to expect return on investment is that payers will be more likely to reimburse efficacy for the targeted population, and not purchase innovative new medications for those individuals with a low risk of success.

## THE COMMERCIAL FOLKLORE OF SEGMENTING PATIENTS vs REALITIES

Why would a drug developer target therapy to only 30–40% of the patients? The tacit commercial assumption is that the drug would actually achieve 100% market share. Yet if real drugs are evaluated by market share, the assumptions about target populations are often woefully unmet. For example, one drug in the GSK portfolio which had > \$1 billion peak sales, achieved up to 14% of the market share at its peak sales. If the 40% of responsive individuals could be accurately identified using PGX tools during development, peak sales equivalent to more than three times the achieved market share could have been targeted much earlier during the commercial life of this drug. The issue of efficacy is in no way diminished when a drug becomes generic, and then there is even less incentive for research investment.

From an economic point of view, discovering and developing innovative safe and effective drugs is the first rule for success but, the real opportunities for companies to succeed also must include satisfying the “ethics” of reimbursement. New drugs that are effective will be reimbursed by HMOs, government agencies, and other payers. They (we) simply do not want to purchase drugs that are not predicted to work—nor do patients want to risk side effects and AEs with drugs from which they do not anticipate efficacy. A multiple-drug market with coverage for all responsive patients may well be the future of pharmaceutical medicine. To anticipate the future, pharmaceutical companies require up to a decade lead time for early discovery projects.

## PRACTICAL CONSIDERATIONS FOR DRUG DEVELOPERS

Both of the examples (APOE and HLA-B\*5701) discussed in this review were applied as ‘known’ PGX markers. Both were discovered before they were applied to pipeline PGX, but were also confirmed as associations during clinical trials (Saunders and Roses, 1993; Corder *et al*, 1993; Hetherington *et al*, 2002; Mallal *et al*, 2002). Thus the role of pipeline PGX can also be viewed as having a drug discovery component, especially identifying associations of candidate genes and pathways, which could then be expanded to target selection. How would a drug developer apply this to the pipeline, especially for neuropsychiatric diseases?

There are several important basic requirements for successful application of pipeline PGX. The first two are obvious and necessary: (1) Appropriate informed consent must be obtained and documented, and (2) Patients must have DNA collected and stored appropriately for regulatory submission. PGX should (3) start with the earliest clinical studies, for safety signals during Phase I, and to generate hypotheses early in phase IIA. The impact of starting with a smaller hypothesis generating trial allows hypothesis testing (as a registerable study) in Phase IIb. The APOE example was a Phase IIb clinical trial that demonstrated not only extensive hypothesis testing of other biomarkers than APOE alleles, but resulted in the customized, genotype-specific PGX Phase III Program design agreed with the regulators (Roses *et al*, 2007a; Risner *et al*, 2006).

Drug discovery is usually based on mechanistic hypothesis. If an exploratory discovery of an association of an SNP is real, it can widen the range of candidate genes and pathways and be associated with efficacy in early Phase II clinical trials. It is possible to test polymorphisms from genes related to all of the proposed hypothesis, as well as every other hypothesis that is discussed in the literature using customized arrays without substantially increasing the time or cost of the analyses by adding SNPs. If only one of ~500 genetic variants is associated, it provides a hypothesis and an expanded pathway that can generate additional candidate genes for the subsequent clinical trials. In addition, SNPs in LD with the associated variant can be used to confirm this region of the chromosome and possibly suggest new genes (Li *et al*, 2008). (4) This pipeline strategy is based on extensive, creative candidate gene lists, not initial genome-wide screening. In this manner statistical significance is corrected for ~500 tests, rather than 500 000 or 1 million tests. Besides, if there is a known linkage association, genome wide scanning can be used locally, with the proposed analyses limited to the chromosomal region of linkage, thus substantially reducing the need for a correction based on the whole genome (Lai *et al*, 1998).

Predictive exploratory discovery can be performed with candidate genes. The most critical difference between pipeline PGX trials and academic studies of disease-related genes is the fact that the gene variants will be studied in a relatively smaller group of well-defined group of patients (Roses, 2008c). The difference is that only the patients who receive the drug can be classified as experiencing efficacy, or not. This is independent of disease gene studies, but may well eventually be demonstrated to provide a pathway definable subset of disease heterogeneity. The large number of patients required for genome-wide associations contrasts significantly with the modest number of patients in a Phase II trial. In addition, there is also the general assurance that if patients respond positively as defined by clinical end points, there will be a subsequent clinical trial with which to test any positive data derived from the first study. In the context of drug registration, successive clinical trials are designed to confirm positive signals and rule out false-positives (Mallal, 2008; Roses, 2007). Storage of DNA allows genome-wide

analyses for additional markers for companion diagnostics once there are thousands of patients enrolled (Roses *et al*, 2007b). From the viewpoint of regulators, the initial 'discovery' of a new association for PGX is exploratory—or 'hypothesis generating.' If that candidate is identified and tested in subsequent clinical trials, that is considered 'hypothesis testing.' And, if the trial demonstrates no patients who improved relative to clinically defined end points, then there is no basis for another clinical trial or PGX analysis. The cost of pipeline PGX is relatively trivial when compared with the cost of the clinical trials—and the costs of drug attrition. PGX does not define the patients: but, rather serves to define those patients who responded with prospectively defined clinical end points. Sequential clinical trials can add to the significance of real associations, or rule out false-positives.

The opportunity in neuropsychiatry is especially large because PGX candidate analysis provides an opportunity to segregate drug responders who may give a mechanistic clue to otherwise obscure phenotypic and genotypic heterogeneity. The use of creative candidate lists that include any and all proposed disease gene hypotheses can serve to identify new mechanistic pathways—as had been the case for Alzheimer's disease, or the molecular basis of abacavir hypersensitivity. With modern genetic tools, derived from the genome, it is now possible to propose peer-defined 'outrageous' hypotheses—at little additional financial or statistical cost. Confirmation of such a hypotheses may also define a new pathway of drug discovery, but also qualify the next clinical trial for hypothesis testing regulatory interpretations and save valuable patent or exclusivity time.

## CONFLICT OF INTEREST

Dr Roses is currently the Director of the Deane Drug Discovery Institute at Duke University which involves consulting with investigators regarding pathways to the discovery of new drugs. For consultative activities involving pipeline pharmacogenetics he has created Cabernet Pharmaceuticals Inc., an S-type (individual) corporation in the State of North Carolina, solely owned by Dr Roses and consulting associates. Cabernet Pharmaceuticals Inc., is a PGX consulting and project management company whose customers are pharmaceutical and biotechnical companies with pharmaceutical pipelines. Dr Roses was formerly Senior Vice President of Genetics Research and Pharmacogenetics in GlaxoSmithKline and a member of the Research and Development Executive Committee for 10 years. Dr Roses was a member of the FDA Science Board from 2003 to 2007 and currently consults for the FDA (gratis). Dr Roses has no affiliation with GSK, but owns GSK stock options, all of which expire in October 2009. In addition, Dr Roses owns stock in his GSK retirement fund worth more than \$50 000, which is being liquidated over the 5-year period after his retirement in October 2007. He has no other stock holdings greater than \$50 000. He is on the Board of Directors of the American Dance Festival and is a Lifetime Iron Duke.

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