Pharmacogenetics to Predict Drug-Related Adverse Events

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ABSTRACT

Identification of reliable markers to predict drug-related adverse events (DRAEs) is an important goal of the pharmaceutical industry and others within the healthcare community. We have used genetic polymorphisms, including the most frequent source of variation (single nucleotide polymorphisms, SNPs) in the human genome, in pharmacogenetic approaches designed to predict DRAEs. Three studies exemplify the principles of using polymorphisms to identify associations in progressively larger genomic regions: polymorphic repeats within the UDP-glucuronosyltransferase I (UGT1A1) gene in patients experiencing hyperbilirubinemia after administration of tranilast, an experimental drug to prevent re-stenosis following coronary revascularization; high linkage disequilibrium within the Apolipoprotein E (ApoE) gene in patients with Alzheimer Disease (AD); and the polymorphic variant HLA-B57 in patients with hypersensitivity reaction after administration of abacavir, a nucleoside reverse transcriptase inhibitor for the treatment of HIV. Together, these studies demonstrate in a stepwise manner the feasibility of using pharmacogenetic approaches to predict DRAEs.

Keywords. Pharmacogenetics; adverse drug reaction; drug-related adverse event; single nucleotide polymorphism; Gilbert’s syndrome; Tranilast; Apolipoprotein E; Alzheimer’s Disease; abacavir; hypersensitivity reaction.

INTRODUCTION

Adverse drug reactions are a significant cause of morbidity and mortality in the United States (Schenkel, 2000), occurring in more than 3% of patients admitted into hospitals in the US and other developed countries (Thomas et al., 2000). Drug-related adverse events (DRAEs) prolong hospital stays and hospital costs dramatically (Rigby and Litt, 2000; Schioler et al., 2001), and they produce significant medical and economic consequences within the community even when they do not result in hospitalization (Haramburu et al., 2000). Identification of reliable markers to predict DRAEs is a continuing goal of the pharmaceutical industry and others within healthcare, particularly if the marker(s) has (have) a high negative predictive value, rapid turn-around, and affordable cost. Pharmacogenetic approaches to identify predictive genetic markers for DRAEs are now feasible because of 4 factors: 1) incremental advances and decreasing costs of high throughput-genotyping; 2) the availability of large numbers of single nucleotide polymorphisms (SNPs) to serve as potential predictive markers; 3) new statistical genetics approaches to analyze SNP data and detect associations with DRAEs in patient cohorts; and 4) the availability of positional information from the human genome sequencing project (Roses, 2002). Application of these pharmacogenetic approaches to DRAEs is being pursued in clinical trials and studies within GlaxoSmithKline R&D (GSK), and the findings from these studies demonstrate that this approach is feasible.

Three studies will be reviewed here, to demonstrate the feasibility of pharmacogenetic approaches that use polymorphisms including SNPs in a high-throughput manner. These studies were chosen from the many performed in GSK, because they illustrate the progressively higher-throughput use of SNPs: from candidate gene approach; to mapping across a larger multigene region; to screening across the whole genome. In the first study, polymorphic repeats in a candidate gene were used to demonstrate the pharmacogenetic basis of a DRAE (Roses, 2002; Xu et al. [unpublished observations]). In the second study, high-density SNP-mapping across a larger genomic region was used to detect associations of a gene to disease, illustrating the principle of high-density SNP approaches (Lai et al., 1998; Roses, 2002). In the third study, whole-genome SNP mapping is being applied to a cohort with a DRAE to confirm and extend initial observations that were made on the basis of polymorphisms within a case control study (Hetherington et al., 2002).

Polymorphic Repeats in the UGT1A1 Gene in Patients Administered Tranilast

Tranilast is a compound that was in phase III trials to determine if the drug prevented restenosis following coronary revascularization. In the trial, approximately 12% of subjects developed transient, reversible hyperbilirubinemia (levels greater than 2 mg per dL). The nature of this DRAE was strikingly similar to the transient benign hyperbilirubinemia that characterizes Gilbert’s syndrome, and this similarity led to the hypothesis that subjects experiencing tranilast-induced hyperbilirubinemia may carry genetic variants which have been associated with Gilbert’s syndrome. For example, Gilbert’s syndrome has been associated with homozygosity for a (TA) 7-repeat element within the promoter region of the
FIGURE 1.—Pre- and posttranilast bilirubin levels in 1,231 individuals from the PRESTO trial. Cases (n = 146) were individuals with serum bilirubin levels greater than 2.0 mg/dl after tranilast administration; controls (n = 1,085) had bilirubin levels equal to or less than 2 mg/dl. Mean and standard error are shown.

UDP-glucuronosyltransferase I (UGT1A1) gene (Bosma et al., 1992, 1995). To test the hypothesis that tranilast-induced hyperbilirubinemia is expressed in patients with this genetic variant, a candidate gene association study was performed. The results showed that in tranilast-treated subjects who were homozygotes for the (TA)7 repeat element, 40% developed hyperbilirubinemia, compared to none of the (TA)7 homozygotes who were treated with placebo. (Figure 1) (Roses, 2002; Xu et al., unpublished observations).

Unfortunately, tranilast failed to demonstrate efficacy in this phase III trial, and so there is no reason to prospectively validate this observation and to determine if polymorphic variants of the UGT1A1 gene (or other measures) will be a useful measure in patient treatment regimens using tranilast. Nevertheless, this result illustrates the principle of identifying polymorphic variants at a candidate gene level to predict DRAEs.

High-Density SNP-Mapping Within a Multigene Region in Subjects with Alzheimer’s Disease

Clearly the above approach was successful because of an educated hypothesis about variants within an appropriate candidate gene. However, application of a pharmacogenetic approach to detect the basis for DRAEs will often require nonhypothesis driven approaches, using polymorphisms that are common and frequent within the human genome. SNPs exhibit these properties and hence are suitable for nonhypothesis-driven approaches in larger genomic regions (Melton, 2003). A second study illustrates the principle of extending SNP-based approaches beyond candidate genes to larger, multigene regions.

The apolipoprotein E4 (ApoE4) allele is a susceptibility gene variant that accounts for a large proportion of late-onset Alzheimer Disease (AD; Saunders et al., 1993; Strittmatter et al., 1993). To test the feasibility of using a high-density SNP association study to detect associations in specified cohorts, a high-density SNP map was made by identifying SNPs within a large, 4-Mb region of chromosome 19 containing the ApoE gene (Lai et al., 1998). When these SNPs were genotyped in subjects with AD, the study showed high linkage disequilibrium (LD; i.e., high strength of association) within the ApoE gene (Lai et al., 1998) and the surrounding area (Figure 2). This result demonstrated the feasibility of using high-density SNP genotyping to detect significant associations within a case control cohort of subjects. Although the trait defining the cohort in this case was a disease (i.e., AD) rather than a DRAE, this finding demonstrates the feasibility of a SNP-based approach in larger genomic regions.

Whole-Genome SNP-Mapping to Detect Genes Associated with Abacavir Hypersensitivity Reaction

The third example demonstrates application of SNP-based pharmacogenetics to the whole genome, in a stepwise approach. The cohort chosen for this study was subjects who developed hypersensitivity reactions after administration of abacavir (Hetherington et al., 2002). Hypersensitivity reaction is a DRAE that occurs in approximately 4% of subjects administered this drug (Hetherington et al., 2001). Because the human leukocyte antigen (HLA) complex on chromosome 6 encodes many genes whose products participate in hypersensitivity reactions, a study was performed to examine polymorphisms within the HLA region in abacavir-treated subjects. Polymorphisms within the HLA-B region occurred with significantly greater frequency in cases who developed hypersensitivity after abacavir, compared to controls who did not develop hypersensitivity (Hetherington et al., 2001). In particular, the polymorphic variants HLA-B57 and TNF-alpha were identified in 46% and 43% (respectively) in cases with the DRAE of hypersensitivity, compared to 4% and 7% (respectively) of controls without this DRAE (p < .0001) (Figure 3). Similar results were reported in another study (Mallal et al., 2002), and these findings have now been replicated by GSK in a second, larger cohort (E Lai et al., unpublished observations).

Interestingly, Caucasians but not African-Americans demonstrated the association between these polymorphisms within the HLA-B region (Hetherington et al., 2001). It is uncertain whether this lack of association within the African-American cohort was due to the smaller sample size of that cohort, or to other factors that include ethnic differences in...
FIGURE 2.—Localized SNP scan around apoE. The distance from apoE was determined by sequencing a cosmid. The $p$ values were calculated for allelic differences using standard chi-squared tests in an affected Alzheimer population of 270 individuals and an age-matched control population of 278 individuals.

Genes associated with specific DRAEs. This illustrates the potential need to perform pharmacogenetic studies in multiple cohorts that have specific DRAEs but differ on the basis of ethnicity, in order to identify a complete profile of DRAE-associated markers that will be predictive for the entire human population taking a drug.

Moreover, the predictiveness of HLA-B polymorphisms accounts for only half of cases with hypersensitivity, even in the Caucasian population (Hetherington et al., 2001). For that reason, the study has now been extended to a larger cohort and a whole-genome SNP-based approach is being used. The results forthcoming should increase the predictive nature of

FIGURE 3.—Association of abacavir related hypersensitivity reactions with markers in the HLA locus. The $p$ values were calculated using Fisher's Exact test statistic to determine whether there was an association between the presence of a given allele and the presence of hypersensitivity in 84 cases and 113 controls. No $p$-values were adjusted for multiple testing. The chromosomal position of the markers is based on the sequence information at The Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/HGP/Chr6/).
associated SNPs to a larger proportion of subjects with hypersensitivity to abacavir.

**SUMMARY**

Together, the results of these 3 studies illustrate that the principle of using pharmacogenetic markers to predict actual DRAEs is feasible. These studies also demonstrate the need to apply these approaches in cohorts of different ethnicities, and to consider a whole genome approach, in order to obtain a profile of markers that will be fully predictive in the global clinical setting.

The goal of this approach will only be fully met, when the cost and speed of detecting predictive markers in an individual subject is appropriate for routine use in a clinical setting; and when the negative predictive value of these markers is high enough to warrant their commonplace use. This goal appears closer with the results of each new study.

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