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Loss-of-function *CYP2C9* variants: finding the correct clinical role for Type 2 diabetes pharmacogenetic testing

Richard W Grant[†] and

Division of General Medicine, Massachusetts General Hospital and Harvard Medical School, 50–59 Staniford St, Boston, MA 02114, USA, Tel.: +1 617 724 3502, Fax: +1 617 724 3544, rgrant@partners.org

Deborah J Wexler

Diabetes Center, Massachusetts General Hospital, Harvard Medical School, 55 Fruit St, Boston, MA 02114, USA

Abstract

Continuing advances in genetic discovery have uncovered several dozen loci that are associated with Type 2 diabetes, including genetic variants that appear to modify responses to commonly prescribed diabetes medications. The use of an individual's genetic information to guide therapy choices raises the possibility of 'personalized medicine', wherein each patient's treatment plan is tailored based on genotype results. However, before such a model of care can be implemented, research is needed to more clearly quantify the association of genetic variation with treatment outcomes and adverse effects. In this article, we review a study examining the association of genetic variation in the cytochrome P450 2C9 enzyme with glycemic response to sulfonylureas in a large cohort of patients with Type 2 diabetes from the Genetics of Diabetes Audit and Research Tayside Study (Go-DARTS).

Keywords

cytochrome P450 enzyme; genetic polymorphisms; personalized medicine; pharmacogenetics; sulfonylureas; Type 2 diabetes

Pharmacogenetic studies generally have one of two primary goals: to elucidate underlying physiologic mechanisms, or to guide clinical care.

While there has been significant progress on the first goal, and there have been some significant advances toward the second, we have yet to realize the promise of personalized medicine for the management of complex chronic diseases such as Type 2 diabetes. Here, we review a study by Zhou *et al.* that begins to bridge the gap between these two goals [1].

Prior research has established that sulfonylureas (a commonly prescribed drug class used to lower blood glucose in patients with Type 2 diabetes) are metabolized by the cytochrome P450 2C9 enzyme, encoded by *CYP2C9*. Furthermore, two specific variants of this gene (*CYP2C9**2, in which an arginine is replaced by a cysteine at position 144; and *CYP2C9**3, in which an isoleucine is replaced by a leucine at position 349) have been found to lead to loss of function of the enzyme, resulting in reduced drug clearance and higher drug levels in individuals with one or both of these allelic variants [2,3]. By examining a group of adult

patients with Type 2 diabetes, Zhou *et al.* sought to determine whether carriers of these loss-of-function alleles would achieve glycemic control more rapidly and persistently when started on sulfonylureas compared with similar patients with the wild-type *CYP2C9*.

This study is one of many from a group of collaborators involved in the Genetics of Diabetes Audit and Research Tayside Study (Go-DARTS) [4,5]. Established over a decade ago, the Go-DARTS cohort has been an excellent resource for evaluating the clinical impact of genetic variation on a relatively large scale. This population-based patient cohort combines clinical and pharmacy prescription data collected within the Tayside region of Scotland from blood samples obtained with informed consent for genetic association studies. This cohort, therefore represents a useful laboratory for linking genetic variation with ‘real-world’ clinical care patterns.

Summary of methods & results

Patient cohort

Patients with Type 2 diabetes were identified from the Go-DARTS cohort. From a total of 3048 incident sulfonylurea users with available genotype results identified in the cohort between 1992 and 2007, 1073 (35%) were eligible for analysis. To be eligible, patients were required to have more than one sulfonylurea prescription, have complete clinical information (e.g., demographic, laboratory and prescription data), baseline hemoglobin A1c (Hb_{A1c}) levels above 7%, and a period of consistent sulfonylurea use of at least 6 months. A key strength of this cohort is the availability of medication adherence measurement (based on pharmacy drug dispensing records).

Exposure

The primary exposure was *CYP2C9* genotype. The advantages of genotype cohort studies are that the exposure (i.e., genotype) by definition always precedes the outcome, and treating clinicians are unaware of genotype status (obviating any concerns about confounding by indication). Using standard methods, the investigators genotyped two small nucleotide polymorphisms (rs1799853 and rs1057910) that correspond to the two loss-of-function allelic variants (denoted *2 and *3). Allelic frequencies were 13.4% for *CYP2C9**2 and 7.3% for *CYP2C9**3. Based on expected Hardy–Weinberg equilibrium, only 49 subjects (4.6%) were homozygous for any combination of the two loss-of-function alleles (i.e., *2/*2 or *2/*3 or *3/*3).

Outcome definition

The authors assessed glycemic response to therapy using three different measures:

- Whether or not subjects reached a Hb_{A1c} less than 7% within 18 months of treatment initiation (a dichotomous outcome);
- A maximal decline in Hb_{A1c} level over 18 months (‘trough’ Hb_{A1c}, a continuous outcome variable);
- Time to monotherapy failure (defined as a requirement for additional treatment).

All outcomes were assessed within multivariate models controlling for baseline differences in patient characteristics.

A challenge in using ‘usual care’ data is to define robust outcomes with data collected for clinical rather than research purposes. Indeed, a substantial number of subjects from the larger incident sulfonylurea user cohort were excluded due to missing prescription data (394/3048; 12.9%), or normal or missing baseline Hb_{A1c} levels (676/3048; 22.2%). The requirement for

at least 6 months of stable sulfonylurea therapy was another major reason for exclusion (771/3048; 25.3%). While this requirement had the advantage of allowing the evaluation of patients who had sufficient exposure to the drug to permit differentiation in treatment efficacy, a major limitation imposed by this criterion is that patients with early adverse reactions are excluded from the cohort. This is of particular relevance since the loss-of-function phenotype, by boosting sulfonylurea levels, could worsen adverse effects such as hypoglycemia, and lead to early discontinuation of the medication.

Main results

The authors successfully demonstrated the predicted link between loss of function of cytochrome P450 2C9 enzyme activity (and, therefore, increased sulfonylurea levels) and improved glycemic control. The 4.6% of patients homozygous for any combination of the two loss-of-function alleles were 3.44-times (95% CI: 1.65–7.15) more likely to achieve treatment target ($p = 0.009$), had a 0.50% (95% CI: 0.18–0.83) greater reduction in baseline Hb_{A1c} levels ($p = 0.003$) and were at decreased risk for monotherapy failure (hazard ratio: 0.79; 95% CI: 0.63–0.99; $p = 0.04$) compared with wild-type controls. Differences between single loss-of-function allele carriers (32.2% of the cohort) and wild-type controls were not statistically significant.

Discussion

This study represents a well-designed and internally valid demonstration of the association between loss-of-function *CYP2C9* variants that decrease sulfonylurea metabolism and several different markers of better sulfonylurea treatment response. While the use of clinical care data from a population-based cohort has important advantages in terms of efficiency of data collection and generalizability of results, this study design also has several weaknesses. For example, because investigators did not themselves measure glycemic response at standard intervals, they used an 18-month window to capture clinical outcomes. This extended window introduces more opportunities for non-genetic modifiers of the outcome. In addition, using 7% as the threshold for treatment failure does not take into consideration cases in which patients have a robust response yet remain above goal. These problems would tend to bias the findings toward the null hypothesis, however, and thus do not limit the successful demonstration of the proof-of-concept. A more significant limitation is the exclusion of the nearly one-quarter of patients prescribed sulfonylureas who did not refill their prescriptions, which precludes any analysis of adverse effects of these drugs. It would have been informative to determine whether the rate of carriers of homozygous loss-of-function mutations was higher in this group. This is important because, in the management of chronic disease, the most useful clinical application of pharmacogenetic information may be in identifying high-risk adverse effects.

What does this study teach us? The primary advance described here is the extension of a known pharmacologic finding initially demonstrated using healthy research subjects (e.g., loss-of-function of cytochrome P450 2C9 enzyme leads to corresponding elevations in sulfonylurea levels) to clinically relevant outcomes (e.g., Hb_{A1c} changes) in a cohort of actual patients undergoing treatment for Type 2 diabetes. Thus, this study represents an important example of how to translate pharmacogenetic advances from the bench to clinical populations.

Do these results have direct clinical utility? Not yet. It is rare that genetic testing modifies the indicated treatment for diabetes. Notably, neonatal diabetes, a monogenic disease that has long been treated as Type 1 diabetes, was found to be due to an activating mutation in Kir6.2, the potassium-sensitive ATP channel [6]; treatment with high-dose sulfonylurea both improved and simplified the treatment of these children, who had uniformly been treated with insulin prior to this discovery [7].

By contrast, in Type 2 diabetes, in which most cases are related to the contribution of many moderate genetic alterations interacting with environmental influences, the marginal benefit of genetic information has been small. In this case, testing patients with Type 2 diabetes for *CYP2C9**2 and *CYP2C9**3 would identify very few patients (~4.6%). While these patients might be expected to receive a modest relative benefit from sulfonylurea therapy, similar clinical results can be discovered more easily by simply initiating the drug.

Expert commentary

As with the use of genetic testing to predict risk for diabetes, in which results have not improved on prediction models that use easily available history, examination and basic laboratory testing [8], the use of single alleles to guide pharmacotherapy is not yet a viable clinical strategy [9]. Let's examine why: unlike the prescription of chemotherapy for a life-threatening malignant tumor (e.g., pulmonary adenocarcinoma, where genetic testing can identify the subset of patients with a specific EGF receptor mutation who will have a rapid and dramatic response to gefitinib [10]), the management of Type 2 diabetes is a chronic process that revolves around effectively teaching and adopting lifestyle changes, and initiating and titrating medications over many years [11]. Medication choices are guided primarily by treatment response, assessed either by Hb_{A1c} results obtained (ideally) at 3-month intervals or, once insulin is introduced, by some combination of Hb_{A1c} testing and self-monitored glucose.

While pharmacogenetic testing may not play a useful role in identifying optimal medication choices in the management of chronic disease, there may be an important role in identifying genetic predisposition to adverse drug effects. Indeed, a number of genetic polymorphisms have been identified in recent years that are associated with increased risk of statin-induced myopathy [12], increased warfarin effect (leading to supratherapeutic levels of anticoagulation) [13], and reduced clopidogrel antiplatelet effect (leading to an increased rate of in-stent thrombosis) [14]. Genetic testing that predicts hypersensitivity reactions to abacavir [15] is now in common clinical use and may be cost effective [16].

Important side effects of treatment with sulfonylureas include hypoglycemia and weight gain. While hypoglycemia is frequently mild in patients with Type 2 diabetes, it may have important ramifications for long-term medication adherence, in addition to causing immediate and potentially serious injury in those who have severe hypoglycemic reactions. Data from recent large clinical trials suggest the possibility that weight gain, which has been tolerated as a necessary evil in the treatment of Type 2 diabetes, may increase cardiac risk more than has previously been recognized [17]. Once the weight has been gained, it is very difficult and costly for sulfonylurea- and insulin-treated patients to lose it. If loss-of-function *CYP2C9* variants predispose to serious hypoglycemia (as might be expected) or excessive weight gain (an untested hypothesis), then the identification of this risk by means of genetic testing prior to the initiation of treatment might more meaningfully guide clinical practice.

Based on the current state of the science, as exemplified by Zhou *et al.*, adding pharmacogenetic testing to current treatment practices is not likely to be a cost-effective addition to the process of medication management for Type 2 diabetes. Rather, what is needed is better public health, better coordination of care and better strategies to improve patient adherence and motivation. Prescribing a medication with confidence that serious adverse reactions can be avoided may promote adherence and treatment success in chronic disease management, as in the case of abacavir and HIV. Furthermore, given the relative cost-effectiveness of patient lifestyle changes [18], an additional role for diabetes genetic testing may be to motivate behavior change in high-risk patients [19].

In summary, work by Zhou *et al.* represents an important first step in linking new pharmacogenetic discoveries to clinical care outcomes. For Type 2 diabetes, a major task is to

identify sub-sets of patients for whom specific drug choices are significantly indicated or, perhaps more importantly, contraindicated. In this respect, the real clinical question is not whether a small number of patients with a relatively rare genotype will do modestly better on a sulfonylurea, but rather whether these patients will be subject to more severe adverse effects, limiting the success of long-term management. Unfortunately, the current study was designed to examine only the first half of this question.

Five-year view

We appear to be on the verge of a new era in clinical medicine [20]. With increasingly well-phenotyped cohorts and innovative genetic analysis methods (e.g., genome-wide association studies, gene copy analyses, DNA methylation and other epigenetic variation), the next 5 years offer great hope for the role of pharmacogenetics to unravel drug and disease mechanisms. The key clinical question is whether this genetic testing approach will ever provide a viable means to individualize pharmaceutical therapy for complex chronic disease [21].

The current gold standard for Type 2 diabetes treatment relies on risk assessment based on easily identified patient characteristics, behavior-change counseling and the timely initiation and adjustment of behavior change and available medications until treatment goals are achieved [11]. For the majority of patients, the incremental knowledge gained from genetic testing is not likely to improve on this current clinical approach. Rather, the real opportunity for pharmacogenetic testing in Type 2 diabetes may reside in identifying the small numbers of patients likely to be harmed by specific medication choices. Given that most patients with Type 2 diabetes are also under concurrent treatment for other common comorbid conditions such as hypertension, dyslipidemia and depression, one could envision a single genetic testing panel based on a large number of relatively uncommon and rare minor alleles that would identify which drugs classes should be avoided in specific patients. The work by Zhou *et al.* is of particular interest in this regard because cytochrome P450 enzymes metabolize such a broad range of commonly prescribed drugs [13,14,22-24].

Key issues

- Pharmacogenetics – the study of associations between genetic variants and drug actions – has been a valuable tool for understanding mechanisms of disease.
- Two cytochrome P450 2C9 enzyme loss-of-function variants (*2 [Arg144Cys] and *3 [Ile359Leu]) have now been associated with both higher sulfonylurea levels and improved glycemic control.
- The rarity of these two variants and their modest clinical impact indicate that there is not yet a clinical role for genotyping to guide diabetes therapy.
- As this field advances, one can envision a future in which a single genotype testing panel that includes a large number of relatively rare genetic variants could be used to guide therapeutic choices for patients with multiple comorbid conditions.

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- of interest
- of considerable interest

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