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SYMPOSIUM

Pharmacogenetics for Type 2 Diabetes: Practical Considerations for Study Design

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Abstract

There is a relative dearth of studies designed to elucidate genetic variation that can explain differences in the response to diabetes pharmacotherapy. When designing such studies, appropriate consideration of the various nongenetic variables that can affect the treatment response is necessary. In addition, disease stage and prior pharmacotherapy also influence drug efficacy. Selecting the appropriate genetic variant to test in such studies is also important, and common variants (known to be functional or otherwise) in a given candidate locus should be tested for the effect on the treatment response. Finally, an appropriate measure of treatment response is necessary to enable detection of pharmacogenetic effects. Perhaps prior to undertaking such studies, smaller studies utilizing well-characterized, homogenous populations with normal glucose tolerance or prediabetes (to avoid the problem of disease effects on treatment response) and surrogate measures of response such as insulin secretion should be completed.

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Introduction

Type 2 diabetes is characterized by defects in insulin secretion and action, which lead to fasting and postprandial hyperglycemia. In addition, the ability of glucose itself to stimulate its own uptake and suppress its own release (glucose effectiveness) is impaired. Defective suppression of postprandial glucagon secretion, as well as abnormalities in gastric emptying, also contribute to the hyperglycemia present in the disease.[1,2]

The transition from normoglycemia to overt diabetes is a gradual process and during this time fasting and postprandial glucose concentrations rise inexorably.[3] However, intensive lifestyle modification can decrease the conversion rate to diabetes, and indeed lifestyle modification is the cornerstone of modern diabetes management.[4] Pharmacotherapy for the disease has witnessed the addition of several new therapies to the treatment armamentarium since the late 1990s.[5] The use of these agents has been tempered by uncertainties regarding their long-term safety, potential side effects, and perhaps variable efficacy.[6]

When considering the potential effect of a drug on glycemic control it is important to appreciate that the drug

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Abbreviations: (DPP) Diabetes Prevention Program, (GLP-1) glucagon-like peptide-1, (HbA1c) hemoglobin A1c, (*KCJN11*) potassium inwardly rectifying channel, subfamily J, member 11, (OCT1) organic cation transporter 1, (*PPARG*) peroxisome proliferator-activated receptor γ, (SNP) single nucleotide polymorphism, (*TCF7L2*) transcription factor 7-like 2

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response may vary depending on compliance (with taking the medication as well as with lifestyle modification), as well as with disease duration, whether the patient is treatment naïve, and also with prior glycemic control. It is usually easier to lower a high hemoglobin A1c (HbA1c) in treatment-naïve patients than it is in a patient with a long-standing history of diabetes who has already been treated with two (or more) medications. Medications that the patient is taking may interact negatively with diabetic medication and also affect the response to treatment. Long-term intervention studies such as the United Kingdom Prospective Diabetes Study have suggested that achieving and maintaining glycemic control become more difficult with time. This is certainly borne out by clinical experience when following patients for an extended period of time.[7,8] Such considerations of patients' treatment history and response are crucial (and need to be accounted for) when selecting a population to assess for variation in drug response.

Genetic Variation, Predisposition to Diabetes and Response to Treatment

The treatment of type 2 diabetes is based on lifestyle modification. Subsequent intervention includes metformin and oral agents such as sulfonylureas and thiazolidinediones. Recent additions to the treatment armamentarium include incretin-based therapies such as glucagon-like peptide-1 (GLP-1) receptor agonists. The latter medications have been associated with a variable degree of weight loss, raising the possibility that genetic variation may underlie the differences in response to such interventions.[9]

Like all complex, multifactorial diseases, type 2 diabetes arises out of a complex interaction between genes and the environment.[10] To date the multiple genetic variants that have been associated with type 2 diabetes are common but have weak effects on disease predisposition; their relevance arises from their implication of pathways in the pathogenesis of disease and suggests avenues of drug development.[11] None of the variants implicated in the pathogenesis of diabetes seem to affect drug metabolism or drug transport to the site of action. However, the first variant to be reproducibly associated with type 2 diabetes, peroxisome proliferator-activated receptor γ (*PPARG*), happens to be the site of action for thiazolidinediones.[12] In addition, the potassium inwardly rectifying channel, subfamily J, member 11 (*KCJN11*), also associated with type 2 diabetes, is the site of action for sulfonylureas.[13] Variation in this locus affected the response to sulfonylurea in 1268 patients as measured by fasting glucose and HbA1c.[14] It has been suggested that transcription factor 7-like 2 (*TCF7L2)* is also associated with variation in the insulin secretory response to GLP-1[15] and with change in HbA1c after the initiation of sulfonylureas.[16]

When considering differences in insulin secretory response to a given therapy, it is important to remember that variants that predispose to diabetes by impairing insulin secretion will be expected to impair the response to a secretagogue (provided these differences are measurable). Likewise, variants that alter insulin action could be expected to impair the response to a sensitizer. In practice, studies examining differences in response to a sulfonylurea attributable to *KCJN11* have focused on the E23K (glutamine to lysine) variant associated with type 2 diabetes. Intriguingly, in the Diabetes Prevention Program (DPP) the variant was associated with an impaired response to metformin.[17] However, it has not been reliably associated with a decreased response to sulfonylureas.[18] The DPP findings underline the fact that it is difficult to dissociate changes in secretion from changes in insulin action especially early in the course of diabetes as both are interrelated with hyperglycemia due to impaired secretion leading to impaired action and vice versa.

As regards the effect of *PPARG* on the response to thiazolidinediones, no effect of the P12A (proline to alanine) variant on response has been reliably determined.[18] One group of investigators has, however, suggested that variation at this locus other than the P12A variant could explain differences in response. These findings were not reproduced in larger studies.[19]

More recently, Shu *et al.*[20] reported that nonsynonymous variation in the organic cation transporter 1 (OCT1), a transporter responsible for the hepatic uptake of metformin (to its putative site of action), decreased the response to two doses of metformin as measured by an oral glucose tolerance test. There are some caveats to this observation, namely that the end point tested is notoriously liable to variation and also it remains to be seen whether chronic dosing with metformin can overcome the putative defect in OCT1-mediated metformin transport. Large intervention studies that utilize metformin, such as the DPP, are ideally placed to answer this question. However, the effect of two loss-of-function polymorphisms in this locus was examined in another study which concluded that the response to metformin was unaffected in 1531 subjects with type 2

diabetes.[21] This contrasts with a small study of 102 patients treated with metformin in whom the rs622342 poly-morphism was associated with HBA1c lowering.[22]

Other than the studies just described, there is a relative dearth of pharmacogenetic studies in type 2 diabetes, and indeed in many other chronic diseases. A candidate gene approach has been used to examine the effect of functional variants in genes that may affect the response to and clearance of sibutramine—a noradrenergic and serotonergic reuptake inhibitor approved for the long-term treatment of obesity.[23] The population studied was relatively small (n = 181) and the duration of treatment was relatively short (12 weeks). However, the population was homogenous and well characterized and a surrogate physiological measure (gastric emptying) was utilized in addition to weight reduction. Functional variants in candidate genes were tested because of the (potential) importance of these candidate genes to modulate sibutramine activity and clearance. Variation at the α2A adrenoceptor, at the serotonin receptor, and at the guanine nucleotide-binding protein β polypeptide 3 (which modulates postreceptor signaling) seemed to significantly affect weight loss induced by sibutramine. Perhaps this study design epitomizes the ideal study design for functional pharmacogenetic studies in diabetes, which to date have been hampered by several limitations.

Selection of Appropriate Measures of Treatment Response

The first requirement of a good study design is selection of a physiologically appropriate end point for drug effect. While HbA1c might appear to be an appropriate end point for an intervention study, it is important to consider whether the duration of the study would be long enough to allow meaningful changes in HbA1c.[22] Similarly, the baseline HbA1c might affect the magnitude of response expected or detectable—it is hardly a sensitive measure in subjects with prediabetes, for example. Other studies have used relatively arbitrary dichotomous end points to define failure or absence of a drug response. Is a random glucose >300 mg/dl truly a good marker of sulfonylurea failure?[24] Is the ability to lower baseline glucose by >20% or baseline HbA1c by >15% a good measure of drug action and, more importantly, do these end points have clinical relevance?[25]

Another important consideration is the methodology used to measure end points. Insulin secretion is not measured by measuring circulating insulin concentrations. Similarly, qualitative measures of insulin action, such as the homeostasis model assessment, which also depend on circulating insulin concentrations, are also potentially misleading, as peripheral insulin concentrations are a function of pancreatic secretion and hepatic clearance. Insulin action as measured by a hyperinsulinemic, euglycemic clamp does not distinguish between hepatic and peripheral insulin action. Furthermore, intravenous glucose delivery may not truly account for drug or drug/gene interactions that alter gastrointestinal motility, absorption, or incretin secretion.

The other consideration is in regards to the genetic architecture of the gene being studied. It is often assumed that the presence of a variant identified by genome-wide association as being associated with a disease is itself the etiological variant. This is rarely, if ever, the case. Indeed, fine mapping of such loci has been undertaken for relatively few loci. For example, the intronic rs730146 single nucleotide polymorphism (SNP) in *TCF7L2* is associated with type 2 diabetes; although not in a coding region, fine mapping has been unable to identify a more associated variant or indeed another variant that can explain predisposition to a disease.[26] In contrast, the sole nonsynonymous SNP in the cytotoxic T lymphocyte-associated protein 4 does not mediate predisposition to autoimmune disease as previously assumed, and fine mapping identified a different variant and a novel mechanism of disease predisposition.[27] The (E23K) nonsynonymous SNP in *KCNJ11*, however, is likely the functional variant in this locus.[17] However, in the case of *PPARG*, it has been suggested that variants other than the P12A may underlie the response to intervention.[19] Therefore, when testing the effect of variation in a locus on a drug response, it is important that the variants of known function be included and that the common variants in the locus are otherwise tested thoroughly. Genetic heterogeneity within the population will also need to be accounted for during the genetic analysis.

A relatively small (n = 73) study by Schäfer *et al.*,[15] and the previously discussed study by Grudell *et al.*,[23] may illustrate some of the principles that may underlie pharmacogenetic studies in the future. The authors studied nondiabetic subjects, thereby avoiding the confounding effects of diabetes on response. The response measured was insulin secretion using a hyperglycemic clamp—an appropriate choice given that the intervention tested (GLP-1) is a secretagogue; although two variants in one locus (*TCF7L2*) were tested, the locus itself was previously well characterized.[28] In such circumstances, results of the study may provide a rationale to examine

the effects of genetic variation in *TCF7L2* in response to incretin-based therapy in diabetic subjects.

Conclusion

Over time, clinical trials examining the effect of drugs on disease have moved their focus away from end points such as low-density cholesterol, HbA1c, and albuminuria to harder end points such as myocardial infarction, cardiovascular events, death, and the development of renal failure. However, the focus on hard end points has necessitated an increase in study size, duration, and complexity. Are pharmacogenetic studies ready for such an evolution? At the present state of knowledge, it is likely unreasonable to design studies of such magnitude and complexity primarily to investigate the role of genetic heterogeneity on drug responses (although suitable provisions for pharmacogenetic substudies, e.g., see the DPP, can be made). A focus on smaller studies with very homogenous populations of patients, who are phenotypically well characterized and who can be subjected to detailed follow-up, may be an important first step. In such situations, the end point measure for the drug response needs to be physiologically relevant to clinical practice and future, larger, population-based studies.

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