Pharmacogenetic Perturbations in Humans as a Tool to Generate Mechanistic Insight

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The field of human genetics has witnessed tremendous acceleration in the last decade, driven in part by the advent of genome-wide association studies (GWAS) (1,2). However, novel insights have not kept pace. In most cases, the specific DNA sequences that cause the molecular changes leading to type 2 diabetes (T2D) have not been identified, and robust signals of association are often initially opaque with regard to a plausible functional mechanism (3,4). Thus, although GWAS constitute a powerful approach to rapidly and systematically uncover associations that may open new windows into T2D pathophysiology, they do not circumvent the need to refine the associated loci to find the precise "causal" DNA sequences—causal in the sense that altering these sequences would eliminate the clinical phenotype (5,6).

We therefore need complementary strategies that both identify the genes of interest within each associated region and illuminate their function. The integration of physiological and pharmacogenetic information with genetic discoveries can provide such a path. By perturbing a live human with a drug that targets a given gene and assessing the response to this perturbation, one may be able to "close the loop" and demonstrate that the gene is indeed involved in producing the phenotype of interest. In a reciprocal fashion, drugs that modulate a specific limb of the glucose homeostatic system (e.g., insulin secretion, central or peripheral insulin sensitivity), if shown to elicit differential responses depending on genotype, may serve to prioritize genes in a given associated region.

The article by 't Hart et al. (7) in this issue illustrates this approach. The authors used the Metabochip to identify genetic variants that influence glucagon-like peptide-1 (GLP-1)–induced insulin secretion in 232 nondiabetic participants from two separate Dutch and German cohorts who were treated with a hyperglycemic clamp. The Metabochip is a custom-designed Illumina array that contains ~200,000 single nucleotide polymorphisms (SNPs) culled from the top regions of the meta-analyzed GWAS distributions for a number of cardiometabolic phenotypes (8). Of these SNPs, 66,000 were selected for efficient replication of top signals across multiple traits, with the hope that when tested in sufficiently large samples they might exceed genome-wide statistical significance (9,10); 120,000 other SNPs were included for the purposes of fine-mapping. Of 53,000 replication SNPs that passed quality control in this study, three SNPs loci exceeded the prespecified experiment-wide statistical significance threshold for association (set at $P < 8.8 \times 10^{-7}$ based on 53,000 SNPs tested): rs4148941 in the 3′ untranslated region of the gene *CHST3*, rs7202633 84 kb upstream of *TMEM114*, and rs7202877 near *CTRB1* and *CTRB2*. For the latter SNP, carriers of the G allele had increased GLP-1–stimulated insulin secretion.

These SNPs were then genotyped in two cohorts of Dutch and Scottish patients with T2D treated with either GLP-1 receptor agonists (22 in the Netherlands and 151 in Scotland) or dipeptidyl peptidase-4 (DPP-4) inhibitors (49 in the Netherlands and 305 in Scotland). For rs7202877, the presence of the G allele seemed to confer relative unresponsiveness to DPP-4 inhibitor treatment, when compared with TT homozygotes.

To elucidate the potential molecular mechanism of action of rs7202877, the investigators assayed expression of the neighboring genes *CTRB1*, *CTRB2*, and *BCAR1* in 35 human pancreata and in islets isolated from 24 normoglycemic and 21 hyperglycemic subjects. In both experiments it appeared that rs7202877 acts as a *cis*-expression quantitative trait locus for *CTRB1* and *CTRB2* but not *BCAR1*. Because *CTRB1* and *CTRB2* encode chymotrypsinogen, chymotrypsin activity was measured in stool samples of 80 participants: consistent with the expression results, 40 G-allele carriers had increased chymotrypsin activity when compared with noncarriers. Stool chymotrypsin activity was reduced after DPP-4 treatment in TT homozygotes, but not in G-allele carriers. Interestingly, the G allele had been previously associated with increased risk of type 1 diabetes (11) but lower risk of T2D (10).

The authors integrated these data in a new and provocative model (Fig. 1). In this model, the G allele at rs7202877 serves as an expression quantitative trait locus for *CTRB1* and *CTRB2*, raising their expression levels and augmenting chymotrypsin activity. This affects the delivery of nutrients to the gut, triggering unexplored changes in the incretin system that cause improved sensitivity of pancreatic β-cells to GLP-1. As a result, G-allele carriers experience higher insulin secretion after an oral glucose load, thereby lowering their T2D risk. The pharmacogenetic findings fit into this model because chymotrypsin is a target for DPP-4 (12). Thus, individuals with higher chymotrypsin levels are relatively resistant to the action of DPP-4 because of their larger "chymotrypsin reserve." If deficits in chymotrypsin activity are crucial for the therapeutic effects of DPP-4 inhibition, G-allele carriers stand to benefit less from the action of DPP-4 inhibitors.

Though appealing, this model poses a number of unanswered questions and raises potentially testable hypotheses. First, what is the mechanism by which increased chymotrypsin activity improves sensitivity to the action of GLP-1

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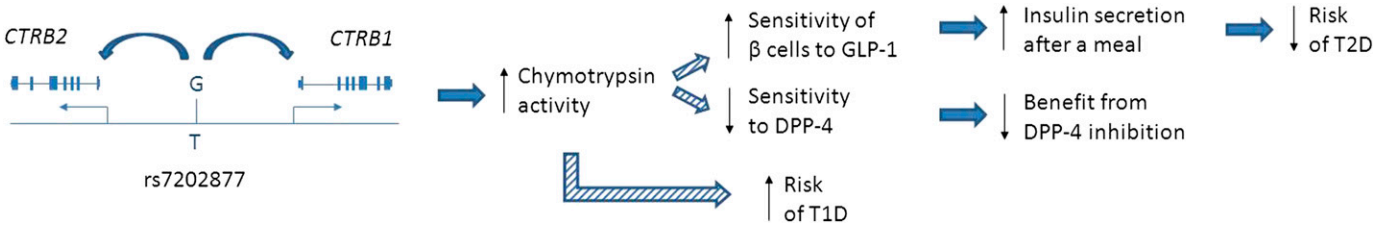

FIG. 1. Diagram depicting the model postulated by the findings in 't Hart et al. (7). The G allele at rs7202877 near the *CTRB1* and *CTRB2* genes raises their expression levels, resulting in higher chymotrypsin activity. Through unclear processes, this improves sensitivity of pancreatic β-cells to the action of GLP-1 while diminishing the individual's sensitivity to DPP-4. Increased incretin sensitivity improves β-cell function (13), thereby lowering T2D risk (10). Because G-allele carriers are less sensitive to DPP-4, they might benefit less from DPP-4 inhibition. In addition, the G allele has been associated with type 1 diabetes (T1D) (11). Hashed arrows indicate steps in the model where mechanistic insight remains speculative (see text for details).

on β-cells? The simplest explanation might be that it modulates GLP-1 levels, yet there is little evidence for changes in circulating GLP-1 levels in three cohorts evaluated in this study. The more compelling alternative, suggested by the GLP-1 infusion data, is upregulation of GLP-1 receptors in either number and/or sensitivity; whether this is indeed the case and how it might take place remains a matter of speculation. Furthermore, the potential hypersensitivity induced by the G allele at rs7202877 is not supported here by the lack of a differential genotype effect of oral glucose or GLP-1 receptor agonists (though the G allele has recently been associated with improved disposition index in a separate human study [13]). Second, the model postulates that increased chymotrypsin activity renders G-allele carriers relatively insensitive to DPP-4 inhibition and therefore less likely to benefit from these agents. However, if the main reason for the effectiveness of DPP-4 inhibition is interference with proteolysis of GLP-1 and prolongation of its half-life (rather than affecting chymotrypsin activity), then it is not clear why its therapeutic action would be contingent on genotype at *CTRB1/2*, given that no differences in GLP-1 levels are noted across genotypic groups. And third, it is not apparent that the mild inhibition of chymotrypsin activity by DPP-4 ($\sim$5%) is large enough to be clinically significant, in either the proposed modulation of the incretin axis or the differential response of G-allele carriers.

A couple of other limitations bear mentioning. As is often the case with complex pharmacological and sophisticated phenotyping studies, the sample size is small by genetic standards. For example, the association of genotype with GLP-1–stimulated insulin secretion was predicated on 36 heterozygotes and 4 homozygotes, whereas the assessment of chymotrypsin activity in response to DPP-4 inhibition was based on 10 G-allele carriers in the Netherlands and 57 G-allele carriers in Scotland. Consequently, the index association exceeds experiment-wide but not genome-wide significance, suggesting that a test of association with all 200,000 SNPs in the Metabochip (rather than the 53,000 replication SNPs that passed quality control) might not have overcome correction for multiple hypothesis testing. Therefore, it would be premature to invoke genotype at this locus as a predictor of clinical response to marketed DPP-4 inhibitors.

Nevertheless, the authors make up for these fixed shortcomings by leveraging several independent cohorts that produce strikingly consistent results. Also in their favor is the previously reported genome-wide association of this locus with T2D risk, substantially raising the prior probability of its involvement in glycemic regulation. How it influences risk of type 1 diabetes, and whether increased chymotrypsin activity increases exposure of pancreatic self-antigens, remain tantalizing questions for further investigation.

In sum, the assembly of state-of-the-art phenotyping, pharmacogenetic, and genetic approaches in humans, once again achieved through international collaboration, has placed an unsuspected pathway on the map for the pathophysiology of T2D. As it often happens with pioneering research, the new results do more to raise intriguing questions than to provide clinically actionable answers. Assuming these findings are confirmed, genotype might be used to stratify patients who are more likely to benefit from DPP-4 inhibitors. Whether delivering chymotrypsin to the gut of T2D risk-allele carriers would aid in improving β-cell function (in either individuals at risk for or with established T2D) would require ensuring that it does not also increase the risk of autoimmune diabetes.

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