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# Type 2 Diabetes Genes Gleaned by Making a β-Cell Screen Routine

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Genome-wide association studies (GWAS) have successfully identified numerous sites associated with type 2 diabetes (T2D) susceptibility (1,2). The vast majority of these sites reside in the noncoding portion of the genome, which does not therefore immediately implicate the culprit gene underlying the association. Making that connection is a key unmet challenge, crucial for studies of genetic mechanisms, designing therapeutics, and clinical decision making. Recent reports have indicated that gene regulation can be coordinated across extensive genomic distances (3); thus, the challenge of linking genetic associations to the corresponding relevant gene(s) has become increasingly daunting. Although a number of functional experimental approaches and studies in model systems are increasingly being used, including in the diabetes field (4–7), these approaches have not yet resolved conclusively the culprit genes at the vast majority of T2D association signals reported to date.

To address this challenge, Thomsen et al. (8) report the results of a high-throughput functional screen they conducted in order to narrow the list and possibly implicate causal genes at T2D GWAS-implicated loci (Fig. 1). The approach—a systematic knockdown of candidate genes via RNA interference, subsequently profiled with physiologically relevant assays—has been limited in the case of T2D for two reasons. First, the availability of appropriate human cell models has proven elusive. Although animal models offer obvious advantages, human cell lines a priori may better reflect the control of the expression of T2D-related genes in the genome. β-Cells are an obvious model system to study signals in T2D pathogenesis, and this study takes advantage of a recently established cell line model for this tissue type, namely, EndoC-βH1 (9). Although imperfect, this line recapitulates insulin secretion in response to glucose or secretagogue stimulation, an important advance in a field that has lacked a model until recently. Second, as a result of using an innovative insulin assay via miniaturization, the authors were able to screen all protein coding genes within 1 Mb of 75 established T2D signals. After corroborating gene expression of the targets in both the cell line and primary β-cells, they were able to reduce that list down to a total of 300 genes, which represented targets that showed evidence of consistent gene expression in this cell setting, at least at the mRNA level. Knowing which genes are ultimately translated consistently at the protein level will need to be the subject of a future study.

The screening criteria used 45 implicated genes across 37 loci. These were collectively derived from two assays, one for cell count and one for insulin secretion, each under varying conditions. As a positive experimental control, genes known to cause maturity-onset diabetes of the young were enriched among their hits, suggesting that biologically relevant genes should be discovered from their approach. The screening for cell count implicated *ZMIZ1*, which was also one of a very few number of genes with defects in both assay settings. A total of 10 of the 35 genes showed hits in two or more conditions, with the tolbutamide experiment, a close proxy to high glucose–stimulating conditions, yielding the most obviously dissimilar results when contrasted with the low glucose–stimulating condition.

To verify that results from the screen were on target, the authors validated eight target genes with newly synthesized small interfering RNAs (siRNAs). *ZMIZ1* was confirmed, along with *ARL15* and *THADA*, albeit that the latter showed weaker evidence in the initial screen. However, all three genes provide new biological insights into β-cell function. In addition, a surprising result was

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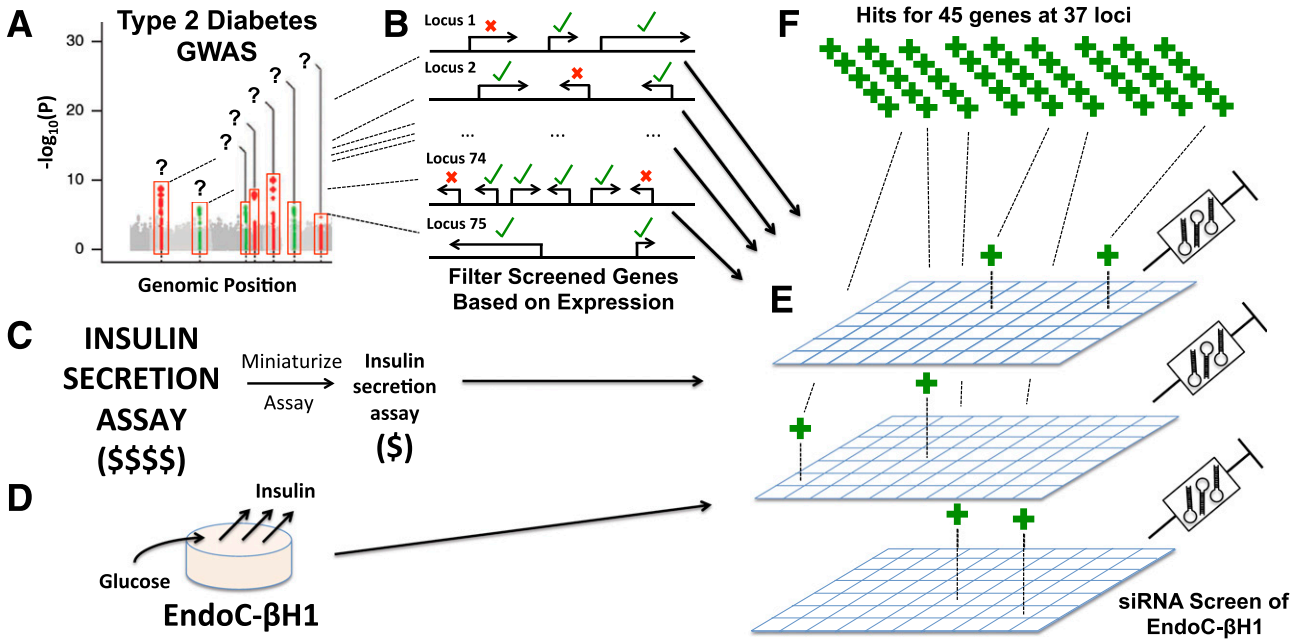


**Figure 1**—Flow diagram of the high-throughput functional screen to implicate causal genes at T2D GWAS-implicated loci. *A*: Seventy-five T2D loci established by GWAS were taken forward, making no assumption as to what the causal genes were at each location. *B*: Corroboration of gene expression within 1 Mb of each given GWAS signal in both the EndoC-βH1 cell line and primary β-cells, resulting in 300 genes deemed eligible for the next steps. Leveraging a cost-effective miniaturization approach to assay insulin (*C*) derived from the EndoC-βH1 cell line (*D*) in order to cross-reference with an siRNA knockdown of the selected genes (*E*), which implicated a total of 45 candidate genes at 37 T2D loci (*F*).

obtained for *HNF4A*, where an increase in insulin secretion was observed following gene silencing. Although the authors speculate as to why this may have come about, this observation could reflect certain limitations to the approach.

Although the results are intriguing, it is important to temper enthusiasm with some limitations of the design, many of which Thomsen et al. (8) acknowledge. First, genes were not considered if the expression was only limited to one of the two β-cell settings, and the authors did experience large variation in the rates of knockdown, which might mask some key effects that they were aiming to identify. Second, screening approaches similar to the one used here will yield false negatives. One possible reason for that would be if loci do not principally operate through β-cell defects and actually involve other physiological tissues of action: adipose, muscle, intestine, liver, or even brain. Indeed, some of the T2D loci are known not to have an obvious correlation with β-cell growth or insulin secretion defects, such as the *FTO-IRX3/5* locus (10). Third, and perhaps most important, although the genes the authors report may be genuine regulators of β-cell function, the genes may have actually nothing to do with the GWAS-implicated signals themselves: by design, the assay does not directly measure variant-to-gene correlation.

This valid screening approach opens the door to larger-scale small molecule or siRNA screening: either to look for rescue or positive cellular responses. Perhaps moving forward, it would be optimal to consider only protein-coding genes in the knockdown in the first instance, subsequently including knockdown of noncoding RNA transcripts. Furthermore, addressing which genes are actually translated to a protein product in the elected cell setting would be another logical point to include in the design. In addition, although knockdowns provide insight, it would have been optimal to also address the impact of overexpression in parallel, which was not part of this study design. Last, multiple gene hits at various loci suggests the possibility of multiple genes operating at a single site (i.e., a locus control region). In fact, the precise genes controlled may differ between cell types, representing an exciting topic for future research in elucidating intricate genetic mechanisms at these sites and optimistically offering even more possible therapeutic avenues to pursue.

With these caveats and future possibilities acknowledged, the importance of the work is transparent: the screen yields excellent T2D candidate genes, genes implicated in β-cell function, and a direct, plausible hypothesis for therapeutic perturbation of the cognate gene (inhibition vs. agonism). By making screens of the β-cell routine, the prospect of identifying causal genes responsible for T2D may be closer than we realize.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

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