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Preview

Genomic discoveries unveil mechanistic insights in diabetes

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Two diabetes-related papers are featured in this issue of *Cell Genomics*. Gardner et al.[1] focus on type 2 diabetes through exome sequencing, and Benaglio et al.[2] employ a functional genomics approach to advance understanding in type 1 diabetes. In this preview, Jose Florez highlights their contribution toward clinical translation of genomics discoveries.

It is often said that human genomics has over-promised and under-delivered.[3] We have witnessed momentous advances including significant investment in technology and resources, the flourishing and complex organization of various disease-oriented international genomics consortia, the substantial engagement of human capital at all career stages, and the assembly of many thousands of human samples with their corresponding phenotypic and genomic data. Yet the plethora of novel genomic findings that have been published over the last 15 years has not fully translated into a complete understanding of pathophysiology, let alone the identification of viable drug targets or tangible benefits in clinical outcomes. One might argue that not enough time has transpired for fundamental genomic discoveries to make it to the clinic; for example, it took 18 years from the discovery of glucagon-like peptide-1 to the approval of the first incretin drug for glycemic control, and an additional 11 years for demonstration of its cardiovascular benefit.[4] It is fair to note that the explosion of discovery promised by more ardent proponents has not yet been realized.

Nevertheless, the many genomic loci that have been robustly and reproducibly associated with disease phenotypes do serve as initial anchors from which intelligent and rigorous follow-up studies can yield mechanistic insight (Figure 1).[5] Two papers in this issue of *Cell Genomics*, one on type 2 diabetes (T2D) and one on type 1 diabetes (T1D) using different approaches, serve as exemplars of this potential path.

In the first study,[1] E. Gardner, J. Perry, and colleagues from the University of Cambridge present a well-powered genome-wide, gene-based analysis for T2D based on whole-exome sequences in 418,436 UK Biobank participants of European descent, of whom 32,374 had T2D. Their dataset covered 18,691 genes, and results were predicated on meeting exome-wide significance ($p < 6.9 \times 10^{-7}$), concentrating on rare, damaging missense variants. Their findings identified "positive controls" at the known diabetes genes *GCK*, *GIGYF1*, *HNF1A*, and *TNRC6B*, but they also identify novel associations for *ZEB2* (large odds ratio [OR] of 5.5 but only 31 observations, so subject to wide 95% confidence intervals), *IGF1R*, and *MLXIPL*. They focus on *IGF1R* based on the known biology and high relevance: they document an association of the same damaging variants with short stature despite higher circulating IGF-1 levels, which is consistent with IGF-1 resistance conferred by receptor mutations leading to compensatory rises in circulating IGF-1. In support of this notion, a Mendelian randomization analysis of 784 common IGF-1-raising variants confirmed the previously observed relationship between higher genetically determined IGF-1 levels and higher T2D risk. This analysis also documented significant heterogeneity in the effect of this genetic instrument on both height and T2D, indicating that variants that primarily raise IGF-1 levels and enhance downstream signaling might have different effects from those that confer IGF-1 resistance and thereby induce a compensatory increase in IGF-1 levels, but with reduced downstream signaling. The biological relevance of the other novel finding with a robust number of observations (*MLXIPL*) is also highlighted, as this gene encodes the carbohydrate response element binding protein.

This compelling paper leverages several strengths. First, the authors choose to focus on coding variation, which allows for straightforward gene-based, genome-wide analyses because the genetic variants can be easily ascribed to specific genes as the functional unit. Second, they implement a clever filter, limiting their inquiry to variants most likely to be damaging. Third, their UK Biobank cohort has decent statistical power, comprising over 30,000 cases with T2D and over 350,000 controls. Fourth, they deliberately constrain population heterogeneity as a way to facilitate interpretation and avoid potential artifacts when examining rare variation, with the unintended but foreseen side effect of once again concentrating on a population of European descent, given the dataset. And fifth, they apply state-of-the-art tools and leverage multiple available genomic resources for their analyses. Their identification of positive controls confirms the adequacy of the approach, validating their novel findings. Among these, the most tantalizing is a highly relevant pathway via IGF-1 signaling that is most consistent with IGF-1 resistance leading to shorter stature and higher T2D risk, with multiple lines of evidence supporting

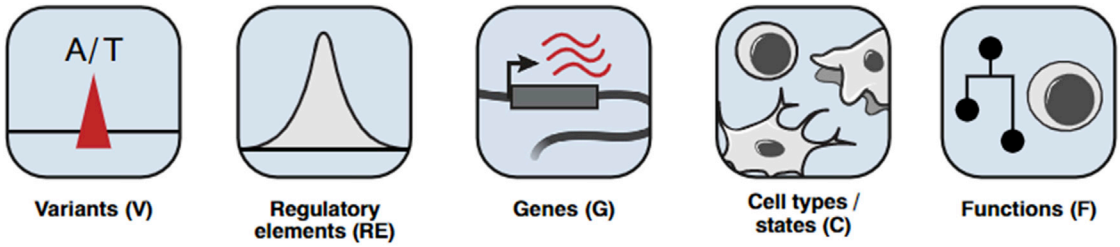

Figure 1. Framework for systematic variant-to-function studies
Reprinted from Claussnitzer and Susztak.[5]

this model and suggesting further functional experiments to help establish a dominant negative mode of transmission. The study introduces a new pathway in T2D pathophysiology and harbors clear translational implications regarding the modulation of the growth hormone axis and T2D risk.

In the second paper,[2] K. Gaulton, M. Sander, and colleagues attempt to identify immune pathways that modify survival of pancreatic β cells in T1D. We have long known that the primary insult in T1D is the autoimmune T cell-mediated selective destruction of β cells. This autoimmune predisposition is denoted by the very large effect size of specific alleles in the *HLA* region, with risk haplotypes conferring ~3- to 10-fold OR of T1D and explaining ~50% of the genetic risk for this condition.[6] However, the remainder of this risk is contained in dozens of polymorphisms of more modest effects, in line with what is seen for other complex traits including T2D. An outstanding question in the field is the molecular mechanisms by which β cells withstand or succumb to the immune attack, which might explain the clinical heterogeneity observed in this disease.

To answer that question, the authors used bulk ATAC-seq to create a map of accessible chromatin in primary human islets cultured in the presence of the cytokines IL-1β, IFNγ, and TNFα across a variety of conditions, yielding a catalog of 165,884 candidate *cis*-regulatory elements (cCREs) responsive to cytokine stimulation. They focused on those with differential responses and used single-nucleus ATAC-seq to home in on β cells, identifying 2,412 cytokine-responsive cCREs in that cell type. To connect them with the genes they regulate in *cis*, they tested both co-accessibility and 3D interactions using HiChIP in the pancreatic β-cell line EndoC-βH1, yielding 2,520 and 2,063 distal co-accessible and interacting cCREs in cytokine-treated and untreated cells; this was complemented by RNA-seq to generate a set of differentially expressed genes. To test the effect of specific genes on β-cell survival after cytokine exposure, the authors then conducted a genome-wide pooled CRISPR loss-of-function screen in EndoC-βH1 cells: this produced 427 genes thought to promote β-cell loss and 440 genes thought to promote β-cell survival, with mitochondrial function emerging as a key modulator of both processes. A subset of these genes, described as 84 pro-survival genes with upregulated expression after cytokine treatment, seemed to be enriched for T1D genomic loci although only at nominal significance; they comprised genes related to modulation of the inflammatory response, ubiquitination, proteasomal degradation, translation, and autophagy. They next used a high-throughput approach to identify 8,424 variants in β-cell cCREs that affect transcription factor binding *in vitro*, of which 2,229 did so in cytokine-response cCREs: T1D-associated variants in β-cell cCREs were enriched for allelic effects on transcription factor binding. The authors further noted that 380 variants in cytokine-responsive β-cell cCREs mapped within 1 MB of a T1D locus and affected transcription factor binding. Given this enrichment between the T1D genetic data and cytokine-responsive β-cell cCREs, these datasets were integrated to produce 77 signals (out of 136) where a variant in the genetic credible set overlapped a β-cell cCRE, of which 52 occurred at β-cell cCREs that were cytokine responsive. The authors use this information to postulate specific effector transcripts at some of the T1D genomic loci where the causal gene had not been definitively elucidated, illustrating this approach with follow-up functional experiments on *SOCS1* as a test case.

This *tour de force* makes several significant contributions to the literature. The generation of a comprehensive map of cytokine-responsive cCREs in β cells is a tremendously useful resource in that it narrows the space for scientific inquiry on the molecular pathogenesis of T1D into a single cell type and under specific immunomodulatory conditions. The incorporation of T1D genomic data is particularly powerful, as it can help select regions where the effect is thought to be causal rather than a consequence of the autoimmune predisposition conferred by the risk *HLA* haplotypes. Finally, the expression data and CRISPR genomic screens can yield select effector transcripts as the biological unit that transduces the genetic risk introduced by the associated causal allele, providing a handle for functional exploration and potential drug target identification. Though appropriate caveats are raised as to the experimental systems employed (three select cytokines and the EndoC-βH1 cell line), this research serves as a general paradigm for the generation of mechanistic insights from genetic studies.

Both diabetes-related papers in this issue successfully illustrate how one can rigorously traverse the laborious path from associated variant to molecular function, beginning to realize the long-awaited promise of genomic discovery.[7]

DECLARATION OF INTERESTS

The author declares no competing interests.

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