Novel genetic findings applied to the clinic in type 2 diabetes

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Genome-wide association studies (GWAS) have both validated known loci and introduced several novel type 2 diabetes genes (table 1; fig. 1). Interestingly, many of the newly discovered variants appear to influence insulin secretion rather than insulin resistance\(^1\). This rapid pace of novel discoveries can elicit a variety of different reactions. By addressing the various questions that arise, I will attempt to place these findings in the appropriate context.

The skeptic may argue that “we have not learned anything new.” Nothing is further from the truth: most of the loci uncovered by GWAS were not in any investigator’s short list of candidate genes, and thus these results have opened new pathways of physiological investigation. Furthermore, areas of the genome of unknown function (e.g. so-called “gene deserts”) have been unquestionably associated with a higher risk of disease, challenging molecular biologists to determine how such genomic regions can have functional effects at the level of the organism. An intriguing link between diabetes and cancer has emerged, where an allele that increases risk of prostate cancer protects from diabetes and vice versa\(^2\), possibly implicating cellular proliferation in the pathogenesis of both diseases\(^3\). Finally, genetic associations have also provided a potential molecular basis for epidemiological observations, as illustrated by the putative involvement of circadian genes in glycemic regulation\(^4-6\).

The absolutist may conclude that “all of the genetic contribution to type 2 diabetes leads to \(\beta\)-cell dysfunction.” While it is true that the heritability of insulin resistance measures is generally lower than that of insulin secretion measures, the former still display a sizeable heritable component\(^7\). Loci discovered via GWAS of type 2 diabetes as a categorical trait are sensitive to study design: when such scans deliberately focus on enrolling leaner cases so as to find genes that cause type 2 diabetes without the mediation of obesity\(^8,9\), their ability to detect genes that increase insulin resistance via adiposity is impaired. Thus, GWAS that intend to discover insulin resistance genes must be designed with that goal in mind, either accounting for the effect of obesity or searching for insulin resistance as a quantitative trait in population cohorts that display enough of a variance in this phenotype\(^1\). It is also possible that the genetic architecture of insulin resistance may differ from that of \(\beta\)-cell function, that our current genotyping arrays do not cover the rele-
TABLE 1. Genetic variants associated with type 2 diabetes at genome-wide levels of statistical significance, ordered by chromosome (Chr)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chr</th>
<th>Description</th>
<th>Gene region</th>
<th>Function</th>
<th>Risk allele</th>
<th>Odds ratio</th>
<th>P value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10923931</td>
<td>1</td>
<td>Intronic</td>
<td>NOTCH2</td>
<td>Transmembrane receptor implicated in pancreatic organogenesis</td>
<td>T</td>
<td>1.13</td>
<td>4.1 × 10^{-6}</td>
<td>11</td>
</tr>
<tr>
<td>rs7578597</td>
<td>2</td>
<td>Missense: T1187A</td>
<td>THADA</td>
<td>Thyroid adenoma; associates with PPAR</td>
<td>T</td>
<td>1.15</td>
<td>1.1 × 10^{-9}</td>
<td>11</td>
</tr>
<tr>
<td>rs4607103</td>
<td>3</td>
<td>38 kb upstream</td>
<td>ADAMTS9</td>
<td>Secreted metallopeptase expressed in muscle and pancreas</td>
<td>C</td>
<td>1.09</td>
<td>1.2 × 10^{-8}</td>
<td>11</td>
</tr>
<tr>
<td>rs4402960</td>
<td>3</td>
<td>Intronic</td>
<td>IGF2BP2</td>
<td>Growth factor binding protein; pancreatic development</td>
<td>T</td>
<td>1.14</td>
<td>8.9 × 10^{-10}</td>
<td>28</td>
</tr>
<tr>
<td>rs1801282</td>
<td>3</td>
<td>Missense: P12A</td>
<td>PPARG</td>
<td>Transcription factor involved in adipocyte development</td>
<td>C</td>
<td>1.19</td>
<td>1.5 × 10^{-7}</td>
<td>29</td>
</tr>
<tr>
<td>rs10010131</td>
<td>4</td>
<td>Intron-exon junction</td>
<td>WFS1</td>
<td>Endoplasmic reticulum transmembrane protein</td>
<td>G</td>
<td>1.15</td>
<td>4.5 × 10^{-8}</td>
<td>28</td>
</tr>
<tr>
<td>rs7754840</td>
<td>6</td>
<td>Intronic</td>
<td>CDKAL1</td>
<td>Homologous to CDK5RAP1, CDK5 inhibitor; islet glucotoxicity sensor</td>
<td>C</td>
<td>1.12</td>
<td>4.1 × 10^{-11}</td>
<td>28</td>
</tr>
<tr>
<td>rs864745</td>
<td>7</td>
<td>Intronic</td>
<td>JAZF1</td>
<td>Transcriptional repressor; associated with prostate cancer</td>
<td>T</td>
<td>1.10</td>
<td>5.0 × 10^{-14}</td>
<td>11</td>
</tr>
<tr>
<td>rs1326634</td>
<td>8</td>
<td>Missense: R325W</td>
<td>SLCOA8</td>
<td>β-cell zinc transporter ZnT8; insulin storage and secretion</td>
<td>C</td>
<td>1.12</td>
<td>5.3 × 10^{-8}</td>
<td>28</td>
</tr>
<tr>
<td>rs10811661</td>
<td>9</td>
<td>125 kb upstream</td>
<td>CDN2A/B</td>
<td>Cyclin-dependent kinase inhibitor and p15 tumor suppressor; islet development</td>
<td>T</td>
<td>1.20</td>
<td>7.8 × 10^{-13}</td>
<td>28</td>
</tr>
<tr>
<td>rs12779790</td>
<td>10</td>
<td>Intergenic region</td>
<td>CDC123-CAMK1D</td>
<td>Cell cycle/protein kinase</td>
<td>G</td>
<td>1.11</td>
<td>1.2 × 10^{-10}</td>
<td>11</td>
</tr>
<tr>
<td>rs7903146</td>
<td>10</td>
<td>Intronic</td>
<td>TCF7L2</td>
<td>Transcription factor; transactivates proglucagon and insulin genes</td>
<td>T</td>
<td>1.37</td>
<td>1.0 × 10^{-40}</td>
<td>30</td>
</tr>
<tr>
<td>rs1111875</td>
<td>10</td>
<td>7.7 kb downstream</td>
<td>HHEX</td>
<td>Transcription factor involved in pancreatic development</td>
<td>C</td>
<td>1.13</td>
<td>5.7 × 10^{-10}</td>
<td>28</td>
</tr>
<tr>
<td>rs5219</td>
<td>11</td>
<td>Missense: E23K</td>
<td>KCNJ11</td>
<td>Kir7.2 potassium channel; risk allele impairs insulin secretion</td>
<td>T</td>
<td>1.14</td>
<td>6.7 × 10^{-11}</td>
<td>31</td>
</tr>
<tr>
<td>rs2237892</td>
<td>11</td>
<td>Intronic</td>
<td>KCNJ1</td>
<td>Encodes the pore-forming α subunit of βK+ channel</td>
<td>C</td>
<td>1.42</td>
<td>2.5 × 10^{-40}</td>
<td>32</td>
</tr>
<tr>
<td>rs7961581</td>
<td>12</td>
<td>Intronic</td>
<td>TSPAN5-LGR5</td>
<td>Cell surface glycoprotein implicated in GI cancers</td>
<td>C</td>
<td>1.09</td>
<td>1.1 × 10^{-9}</td>
<td>11</td>
</tr>
<tr>
<td>rs8050136</td>
<td>16</td>
<td>Intronic</td>
<td>FTO</td>
<td>Alters BMI in general population</td>
<td>A</td>
<td>1.17</td>
<td>1 × 10^{-12}</td>
<td>28</td>
</tr>
<tr>
<td>rs757210</td>
<td>17</td>
<td>Intronic</td>
<td>HNF1B</td>
<td>Transcription factor involved in pancreatic development</td>
<td>A</td>
<td>1.12</td>
<td>5 × 10^{-6}</td>
<td>28</td>
</tr>
</tbody>
</table>
The pessimist may announce, “these genetic effects are so small that they cannot possibly be clinically relevant”. Here, it should be noted that effect sizes computed as allele frequency differences between cases and controls say nothing about biological or clinical relevance. The evolutionary constraints imposed by natural selection may be expected to prevent strongly deleterious mutations from rising to high frequencies in the population; but genetic variants that have modest effects on human physiology may indeed shed light on specific molecules or pathways which could be targeted for therapeutic intervention. This concept is illustrated by two polymorphisms of very modest effects (PPARG P12A and KCNJ11 E23K) which lie in genes that encode targets for routine anti-diabetic medications, thiazolidinediones and sulfonylureas respectively.

In another relevant example, a polymorphism in the gene that encodes HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase explains a small proportion of the variance in LDL-cholesterol\textsuperscript{12}; but this minor effect does not imply that HMG-CoA reductase is not an adequate target for LDL-cholesterol lowering, and suggests that this validated target for statin therapy would have been identified by GWAS even if nothing had been known about its mode of action.

The optimist, in turn, may naively proclaim that “the variants identified will be useful in individual clinical prediction”, heralding a quick and successful implementation of personalized medicine. While these discoveries may indeed illuminate biology and highlight opportunities for therapeutic intervention, their clinical use as risk factors in diabetes prediction is much less clear. Current simple clinical tools developed to predict risk of type 2 diabetes perform quite well, with an area under the receiver-operator characteristics curve as high as 85-90\%\textsuperscript{13}. Recent publications evaluating the ability of a genotype score composed of an aggregate of known risk variants to predict diabetes prospectively have shown marginal, clinically insignificant improvements over routinely tested risk factors\textsuperscript{14,15}. The genotype score improved its predictive power when applied to subjects under 50 years of age, allowing for 12\% of this group to be “correctly” reclassified into a high-risk group. This supports the notion that genetic factors may be useful in early detection of at-risk groups before clinical risk factors such as obesity or hyperglycemia manifest themselves, allowing practitioners to recommend effective long-term preventive interventions at earlier stages.

Finally, the pragmatist simply asks whether “genetic information will help guide therapeutic decisions”. While genetic data has proven invaluable in the treatment of monogenic forms of diabetes, such as MODY (maturity onset diabetes of the young)\textsuperscript{16} and neonatal diabetes\textsuperscript{17}, the pharmacogenetics of complex disease is still very much in its infancy. In a retrospective study, Pearson et al showed that patients with the risk variants at TCF7L2 were more likely to fail sulfonylurea therapy than metformin\textsuperscript{18}. The lifestyle intervention of the Diabetes Prevention Program has been particularly effective in carriers of the risk alleles at TCF7L2\textsuperscript{19} and ENPP1\textsuperscript{20}. In contrast, the PPARG P12A variant does not seem to impact the individual response to thiazolidinediones\textsuperscript{21-23}. In one of the few prospective studies published to date, carriers of the risk Ala allele at ABCCS A1369S showed a heightened response to sulfonylurea therapy, a finding that must be replicated\textsuperscript{24}. Examination of drug metabolism genes may also prove fruitful: the OCT1 transporter responsible for metformin uptake harbors variants which influence the human response to an oral glucose tolerance test\textsuperscript{25}. In sum, whether this emerging body of genetic knowledge will direct response to different classes of therapeutics must be empirically tested.

In conclusion, widespread clinical genetic testing for common variants associated with type 2 diabetes is premature\textsuperscript{26}. It is not yet clear that any single variant or a set that includes all of them can predict diabetes onset at the individual level. Furthermore, the impact of this genetic knowledge on the response of patients or clinicians has not been formally tested: it is quite possible that a negative test may provide false reassurance and discourage healthy behaviors. Until such testing demonstrates a beneficial effect on outcomes and is proven to be cost effective, it should only be conducted in the setting of clinical trials.

A number of genetic variants have already been reproducibly associated with type 2 diabetes; the list is

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**Fig. 1.** Type 2 diabetes-associated loci, plotted by year of definitive publication and approximate effect size. Genes implicated in type 2 diabetes by functional and genetic evidence but short of genome-wide significance are shown at the bottom.
only expected to grow. As large datasets of genome-wide data become available, distinguishing true associations from spurious findings due to statistical fluctuations will be essential to guide future work. Testing novel associations prospectively, measuring their precise effects on glycemic traits and assessing whether they affect response to therapy is a key step in their experimental validation. Thus, it is crucial to harness this novel genetic knowledge so that it can refine our understanding of the pathophysiology of diverse forms of diabetes, enhance our prognostic ability and direct our choice of appropriate therapies. The discovery of diabetes, enhance our prognostic ability and direct understanding of the pathophysiology of diverse forms this novel genetic knowledge so that it can refine our conditions from spurious findings due to statistical fluctuations will be essential to guide future work. Testing novel associations prospectively, measuring their precise effects on glycemic traits and assessing whether they affect response to therapy is a key step in their experimental validation. Thus, it is crucial to harness this novel genetic knowledge so that it can refine our understanding of the pathophysiology of diverse forms of diabetes, enhance our prognostic ability and direct our choice of appropriate therapies. The discovery of diabetes, enhance our prognostic ability and direct understanding of the pathophysiology of diverse forms

## Conflict of interest

Jose C. Florez has received consulting honoraria from Merckz, Pfizer, bioStrategies, XOMA and Publicis Healthcare Communications Group, a global advertising agency engaged by Amylin Pharmaceuticals.

## REFERENCES

1. Florez JC. Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: Where are the insulin resistance genes? Diabetologia. 2008;51:1100-10.


