Modelling Type 2 diabetes GWAS candidate gene function in hESCs

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Abstract

Type 2 diabetes is a complex polygenic disorder that affects about 1 in 12 adults. Here, Zeng et al. (2016) elegantly combine CRISPR-based gene editing in hESCs with directed β cell differentiation to investigate the functions of genes highlighted by genome-wide association (GWA) studies for this disease.

Main text

The advent of GWAS more than a decade ago has led to an ever more detailed understanding of the genetic landscape of many complex diseases, including type 2 diabetes (Fuchsberger et al., 2016). Thus, more than 90 *loci* have been identified, with variants in the vast majority affecting the release of insulin. However, in only a handful of cases have attempts been made to understand how variants at implicated *loci* act at the cellular or organismal level. Implicated variants, which include single nucleotide polymorphisms (SNPs), insertion/deletions (indels) and copy number variants (CNVs), often lie in non-coding regions, i.e. in introns or intergenic regions. Consequently, it is usually not possible to determine with certainty which gene in a given locus is likely to mediate altered disease risk, nor the mechanisms through which it/they may act (Rutter, 2014). Functional genomic approaches, whereby the impact of modifying the expression of implicated genes is explored through interventional approaches in the disease-relevant cell type, are usually required to explore these questions.

Whilst gene knockdown in mice or in relevant cell lines have been the gold standard approach for studying gene function (Rutter, 2014), both strategies have limitations. The use of rodents is expensive and may be criticised as not necessarily reflecting changes which occur in humans. On the other hand, developmental processes are difficult to study in cell lines. In type 2 diabetes, most GWAS genes appear to act through insulin-secreting pancreatic islet β cells. Here, Zeng et al (2016) first performed CRISPR-mediated editing in human embryonic stem cells (hESCs) of candidate genes identified from Type 2 diabetes GWAS. The authors then explored the functional impacts of deleting these genes on β cell function by directed differentiation of these lines towards a β cell fate (Pagliuca et al., 2014; Rezania et al., 2014). Further, they identified a small molecule which could rescue the observed disease-associated phenotypes associated with one of the genes.

The authors chose to assess the importance of genes from three distinct *loci* where possible biological roles have been established. Firstly, *CDKAL1* has been shown to be important in β cells both for energy generation (Ohara-Imaizumi et al., 2010) and as a regulator of tRNA

function (Wei and Tomizawa, 2011). Secondly, *KCNQ1* encodes a voltage-gated K^+ channel largely expressed in the intestine and heart and whose inactivation in mice impairs insulin action. However, the role of this gene in controlling insulin secretion is more controversial. Variants at this locus have been suggested to act largely via a neighboring imprinted gene that produces the non-coding RNA *Kcnq1ot1,* which in turn controls the expression of the cyclin-dependent kinase inhibitor 1C (*Cdkn1c*). Thirdly, *KCNJ11* encodes the pore-forming (Kir6.2) subunit of the ATP-sensitive K^+ channel complex (K_{ATP}), and has a coding variant (E23K) linked to monogenic forms of type 2 diabetes (permanent neonatal diabetes) (Gloyn et al., 2004), and is also located close to a diabetes risk SNP (Zeggini et al., 2007).

The authors engineered disruptive mutations in the above genes into a hESC line (HES3G) expressing GFP under the control of the insulin promoter to simplify purification of differentiated β cells. Multiple hESC clones were made for each gene to minimise risks of random variation contributing to an observed phenotype.

Inactivation of none of the three genes impaired the directed differentiation process towards near-normal β cells, arguing against roles in development. However, *CDKAL1*-null cells showed defective glucose-stimulated insulin secretion, and this defect was even more marked in cells deleted for *KCNQ1* or *KCJN11*. In the case of the K^+ channels, the loss of a hyperpolarising current is expected to lead to permanent and glucose-insensitive depolarisation; indeed, activating mutations in the *KCNJ11* gene lead to hyperinsulinemism in man (Nessa et al., 2016). Consistently, insulin secretion under basal (non-stimulated) conditions was increased in cells null for this gene. By contrast, deletion of *KCNQ1* or *CDKAL1* eliminated insulin secretion stimulated by high glucose, whilst responses to cell depolarisation were preserved in *CDKAL1* (but not *KCNQ1* or *KCNJ11*) null cells. In line with these *in vitro* deficiencies, transplanting mutant cells into mice rendered diabetic with streptozotocin only partially corrected hyperglycemia, with *KCNJ11* cells being the least effective of the three lines.

Since an important goal of identifying genes responsible for altered risk of type 2 diabetes is to develop personalized, i.e. genotype-dependent, therapies, Zeng et al used a high content screen and a complementary bioinformatic approach to identify pathways which may be altered by *CDKAL1* deletion. The statistical confidence ($P \sim 10^{-11}$) in *CDKAL1* as a type 2 risk locus is particularly high (Zeggini et al., 2007), making this an attractive choice for therapeutic targeting. T52244, a known small molecule inhibitor of the FOS/JUN activator complex AP1, was identified as substantially preventing apoptosis in *CDKAL1*-null cells. Correspondingly, analysis of RNASeq data revealed that the FOS/JUN pathway, a key regulator of cell growth, was up-regulated in $CDKAL1^{-/-}$ cells. Inhibition of this pathway either through CRISPR-mediated inhibition of FOS or treatment with T52244 rescued the inability of CDKAL1 null cells to correct glycemia in streptozotocin-diabetic mice.

These studies are thus exciting since they move from the identification of risk alleles via population-based GWA studies in man, to cellular work in disease-relevant tissue, and finally to the identification of a novel therapeutic approach. Of course, important questions remain. For example, how does CDKAL1 control AP1 activity? None of the previous studies examining the role of CDKAL1 in mice or in cells have hinted at such a role and suggest a pleiotropic action of this protein. Although clinical trials will be needed to determine whether CDKAL1 represents a tractable therapeutic target for diabetes – and whether the subtle (~15 % per allele; (Zeggini et al., 2007) effect of variants at this locus on diabetes risk will allow genuinely personalized treatment – the present studies undoubtedly represent an important advance and a new paradigm for the study of GWAS genes by functional genomics.

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