Translating *TCF7L2*: from gene to function

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Abbreviations

GIP Glucose-dependent insulintropic polypeptide
GLP1 Glucagon-like peptide 1
SNP Single nucleotide polymorphism

We are living through a revolution in genetics research. At present, barely a week goes by without a flurry of reports revealing robustly replicated loci associating with a common complex disease. Diabetes was one of the first disease areas to yield results from these genome-wide association studies. Prior to 2006, only two genes were established type 2 diabetes loci (*PPARG* [1] and *KCNJ11* [2]). With the latest round of meta-analyses there are 18 such loci [3, 4], probably more by the time this article goes to print. The majority of these new loci are in gene regions that were not previously candidates for diabetes. Thus, unravelling these new pathways could potentially reveal new disease mechanisms and new therapies. However, herein lies the difficulty. In this commentary the genetic discovery of *TCF7L2* is used as a case study to highlight the challenges that lie ahead in terms of defining the functional and therapeutic impact of diabetes susceptibility genes.

Polymorphisms in *TCF7L2* that are strongly associated with diabetes risk were identified under an area of linkage on chromosome 10 by the deCODE investigators, who are based in Iceland [5]. This association has since been replicated across multiple ethnic groups, and with a per-allele relative risk of 1.4, is the strongest association among the common type 2 diabetes genes [3]. The protein product of *TCF7L2* (transcription factor 7-like 2, also referred to as

transcription factor 4 or TCF4) is a transcription factor that is involved in the WNT signalling pathway. Importantly, at the time of the deCODE discovery, the role of *TCF7L2* in the regulation of the glucagon gene (*GCG*), which encodes glucagon-like peptide 1 (GLP1) in the gut L cells, had just been described [6]. This immediately implicated *TCF7L2* in incretin release or incretin signalling pathways, and an impaired incretin system as the mechanism whereby *TCF7L2* polymorphisms increase diabetes risk. However, whilst to some extent supporting this initial hypothesis, studies to determine the mechanism whereby *TCF7L2* is implicated in diabetes remains far from clear, as is highlighted in the text box.

Mechanisms whereby altered *TCF7L2* production or function may cause diabetes

- Decreased beta cell mass
Decreased beta cell proliferation following *TCF7L2* knockdown [12]
Decreased 'maximal' insulin secretion—response to arginine at 28 mmol/l glucose [10]
- Impaired insulin processing or release
Altered *SLC30A8*, *Munc18-1* and *Stx1A* expression in mice with *Tcf7l2* knockdown [13]
Increased proinsulin:insulin ratio in rs7903146 T allele carriers [9, 17]
Decreased 'maximal' insulin secretion—response to arginine at 28 mmol/l glucose [10]
- Impaired GLP1 signalling in beta cells [9, 11]
Response to GLP1 impaired to greater extent than that to i.v. glucose [10]
- Decreased glucagon secretion reducing beta cell insulin secretion [9]
Needs replicating
- Hepatic insulin resistance [9]
Needs replicating

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TCF7L2, incretins and the beta cell

Initial studies revealed impaired insulin secretion in response to oral glucose ingestion in carriers of the diabetes risk allele (T) at the *TCF7L2* single nucleotide polymorphism (SNP) rs7903146 [7, 8]. In this issue of *Diabetologia*, Pilgaard et al. [9] show that T allele carriers have impaired post-meal beta cell responsiveness, i.e. the amount of insulin secreted at a particular glucose concentration is reduced; this is a measure of the beta cell response not just to glucose but also to amino acids and incretins. Lyssenko et al. [10] showed that the insulin secretory response to oral glucose ingestion was greater than that to intravenous glucose in carriers of the T allele at rs7903126. Although, to date, no results have been published on the gold standard assessment of the incretin effect (the isoglycaemic infusion, which involves the intravenous infusion of glucose to achieve plasma glucose levels that match those reached following oral glucose ingestion), this study established that *TCF7L2* variants somehow impair this effect.

What is the mechanism for this reduction? Surprisingly, despite the role of *TCF7L2* in the transcriptional regulation of *GCG*, neither glucose-dependent insulinotropic polypeptide (GIP) [10] nor GLP1 secretion is reduced after oral glucose ingestion in carriers of the risk allele. So rather than directly reducing incretin secretion, *TCF7L2* must play a role in the pancreatic insulin secretory response to incretins. Pilgaard et al. [9] add to the report by Schafer et al. [11] that the insulin secretory response to GLP1, in this study at high physiological concentrations and at mild hyperglycaemia (7 mmol/l), is reduced in normoglycaemic carriers of the T allele at rs7903146. The marked effect of the *TCF7L2* variant on the response to incretins contrasts with the minimal effect on the direct response to glucose and suggests a functional beta cell defect rather than, or in addition to, a loss of beta cell mass.

Two recent in vitro studies on human and mouse islets show strong parallels with the human in vivo work. Reducing *TCF7L2* protein production using siRNA led to a small reduction in glucose-induced insulin secretion and a much greater reduction in GLP1-induced secretion [12, 13]. In the first study [12], knockdown of *TCF7L2* in human islets decreased beta cell proliferation, and overexpression of *TCF7L2* had a protective effect against glucose- and cytokine-induced apoptosis, suggesting a role in regulation of beta cell mass. However, the second study points to a functional defect [11]. Knockdown of *Tcf7l2* in mouse islets primarily affected the expression of genes involved in the late stages of insulin secretion. Notably, there was a reduction in the expression of *Slc30a8*, an established diabetes gene [14], which encodes a zinc transporter that is located in the secretory vesicles. In addition, *Tcf7l2* knockdown reduced the expression of *Munc18-1* (also known as *Stxbp1*)

and increased the expression of *Stx1a* (which encodes syntaxin 1a), both of which encode key players in the control of insulin exocytosis. Interestingly both *Munc18-1* and *Stx1a* contain a putative *TCF7L2* binding site in their 5' untranslated regions, suggesting a direct regulatory role of *TCF7L2* [13]. It should be noted, however, that levels of *TCF7L2* mRNA [10] and *TCF7L2* protein [15] are increased, not decreased, in human islets from type 2 diabetic donors. In addition, T allele carriers had increased islet levels of *TCF7L2* mRNA compared with individuals with the CC genotype [10]. The role of the intronic variant rs7903146 in *TCF7L2*, or the variant it is tagging, and how it relates to these in vitro studies has yet to be determined.

The in vivo and in vitro data support a role for *TCF7L2* in the distal regulation of insulin secretion. What remains uncertain is whether the striking incretin effect reflects a GLP1/GIP-specific effect, or whether this is a generalised defect in insulin processing and secretion. In favour of the latter, *TCF7L2* expression affects the direct effect of glucose both in vitro [12, 13] and in vivo [16]; there is an increase in the proinsulin:insulin ratio in carriers of the risk allele [17], suggesting a defect in insulin processing; and the 'maximum' insulin secretory capacity, as assessed by the response to arginine at a constant plasma glucose concentration of 28 mmol/l, is reduced in risk allele carriers with impaired glucose tolerance or type 2 diabetes [10]. It would be interesting to see how the insulin secretory response to tolbutamide is affected in islets with reduced *TCF7L2* expression, or, indeed, in humans carrying the *TCF7L2* risk allele, as this may help further address this question.

Glucagon concentrations are elevated in patients with type 2 diabetes. In contrast, Pilgaard et al. report that 24 h glucagon concentration profiles are reduced in carriers of the diabetes rs7903146 risk allele [9]. They also report unpublished data for 580 individuals showing decreased plasma glucagon expression with other *TCF7L2* SNPs, but not the established diabetes SNP rs7903146. However, these data contrast with previous reports of normal fasting glucagon in T allele carriers [10] and the finding from in vitro studies that *TCF7L2* does not play a role in *GCG* gene expression in alpha cell lines [6]. *TCF7L2* expression in human islet alpha cells has not been described, and further in vivo replication is required, yet reduced paracrine glucagon stimulation of beta cell insulin secretion would certainly provide another mechanism for decreased insulin secretion in *TCF7L2* risk allele carriers.

TCF7L2 and insulin action

Finally, the paper by Pilgaard et al. [9] introduces a new twist to the story. Euglycaemic-hyperinsulinaemic clamp

studies have shown normal whole body insulin sensitivity [10, 11]. An increase in basal endogenous glucose output has been previously described [10] and attributed to reduced pancreatic insulin secretion. Pilgaard et al. show that carriers of the risk allele of rs7903146 show a reduced suppression of endogenous glucose output in response to both low-dose insulin ($10 \text{ mU m}^{-2} \text{ min}^{-1}$, not statistically significant) and high-dose insulin ($40 \text{ mU m}^{-2} \text{ min}^{-1}$), despite reduced glucagon concentrations, suggesting that these individuals have insulin resistance in the liver [9]. It is important to interpret the results of Pilgaard et al. with caution. Only 34 participants were studied, and these were selected from a study that was designed to investigate the effect of birthweight on the development of type 2 diabetes, although all analyses were adjusted for birthweight difference. If real, the result is intriguing and offers up a number of possible explanations. First, GLP1 acts on the liver to decrease endogenous glucose output [18]. As GLP1 is secreted by the L cells of the gut, the postprandial portal vein concentrations of GLP1 are twice that of the systemic circulation [19]. Thus, GLP1 secretion may indeed be reduced in carriers of the T allele at rs7903146, but this is not detectable at the levels measurable in the systemic circulation. Although not significant, GLP1 secretion in response to oral glucose ingestion was reduced in the T allele carriers in this group [9]. Another possibility is that *TCF7L2* is expressed in the liver [20], and thus may play a direct role in mediating insulin action. Third, this isolated hepatic insulin resistance bears a striking similarity to that described for patients with MODY resulting from *TCF2* (also known as *HNF1B*) mutations [21] via a mechanism that has yet to be determined. Clearly, before postulating further mechanisms, replication of this novel finding by Pilgaard et al. is key.

In summary, it is 3 years since the identification of *TCF7L2* as a key player in diabetes aetiology, yet we still do not know how *TCF7L2* variation causes diabetes. Definite progress has been made as a result of human and in vitro studies, but we are still some way from being able to translate the genetic discovery through to clinical benefit. However, there is no doubt that each step along this path is providing considerable insight into the physiology and pathophysiology of glucose regulation and other pathways. And this is just for one risk variant. With at least 17 other risk variants, there are many more discoveries to be made.

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References

- Altshuler D, Hirschhorn JN, Klannemark M et al (2000) The common PPAR γ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80
- Gloyn AL, Weedon MN, Owen KR et al (2003) Large-scale association studies of variants in genes encoding the pancreatic β -cell K_{ATP} channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572
- Frayling TM (2007) Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev* 8:657–662
- Zeggini E, Scott LJ, Saxena R et al (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 40:638–645
- Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323
- Yi F, Brubaker PL, Jin T (2005) TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β -catenin and glycogen synthase kinase-3 β . *J Biol Chem* 280:1457–1464
- Florez JC, Jablonski KA, Bayley N et al (2006) *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241–250
- Saxena R, Gianniny L, Burt NP et al (2006) Common single nucleotide polymorphisms in *TCF7L2* are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 55:2890–2895
- Pilgaard K, Jensen CB, Schou JH et al (2009) The T allele of rs7903146 *TCF7L2* is associated with impaired insulinotropic action of incretin hormones, reduced 24 h profiles of plasma insulin and glucagon, and increased hepatic glucose production in young healthy men. *Diabetologia* doi:10.1007/s00125-009-1307-x
- Lyssenko V, Lupi R, Marchetti P et al (2007) Mechanisms by which common variants in the *TCF7L2* gene increase risk of type 2 diabetes. *J Clin Invest* 117:2155–2163
- Schäfer SA, Tschritter O, Machicao F et al (2007) Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (*TCF7L2*) gene polymorphisms. *Diabetologia* 50:2443–2450
- Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J, Maedler K (2008) Transcription factor 7-like 2 regulates β -cell survival and function in human pancreatic islets. *Diabetes* 57:645–653
- da Silva Xavier G, Loder MK, McDonald A et al (2009) *TCF7L2* regulates late events in insulin secretion from pancreatic islet β -cells. *Diabetes* doi:10.2337/db08-1187
- Sladek R, Rocheleau G, Rung J et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881–885
- Lee SH, Demeterco C, Geron I, Abrahamsson A, Levine F, Itkin-Ansari P (2008) Islet specific Wnt activation in human type II diabetes. *Exp Diabetes Res* 2008:728763
- Palmer ND, Lehtinen AB, Langefeld CD et al (2008) Association of *TCF7L2* gene polymorphisms with reduced acute insulin response in Hispanic Americans. *J Clin Endocrinol Metab* 93:304–309
- Loos RJ, Franks PW, Francis RW et al (2007) *TCF7L2* polymorphisms modulate proinsulin levels and β -cell function in a British European population. *Diabetes* 56:1943–1947
- Prigeon RL, Qaddusi S, Paty B, D'Alessio DA (2003) Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. *Am J Physiol Endocrinol Metab* 285:E701–E707

19. Dardevet D, Moore MC, DiCostanzo CA et al (2005) Insulin secretion-independent effects of GLP-1 on canine liver glucose metabolism do not involve portal vein GLP-1 receptors. *Am J Physiol* 289:G806–G814
20. Cauchi S, Meyre D, Dina C et al (2006) Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 55:2903–2908
21. Brackenridge A, Pearson ER, Shojaaee-Moradie F, Hattersley AT, Russell-Jones D, Umpleby AM (2006) Contrasting insulin sensitivity of endogenous glucose production rate in subjects with hepatocyte nuclear factor-1 β and -1 α mutations. *Diabetes* 55:405–411