TCF7L2 controls insulin gene expression and insulin secretion in mature pancreatic β-cells

Merewyn K. Loder1,2, Gabriela da Silva Xavier1, Angela McDonald and Guy A. Rutter
Imperial College, Faculty of Medicine, Department of Cell Biology, Sir Alexander Fleming Building, Exhibition Road, South Kensington, London SW7 2AZ, U.K.

Abstract
Genetic studies have linked the risk of Type 2 diabetes with SNPs (single nucleotide polymorphisms) in the gene encoding the Wnt signalling-associated transcription factor, TCF7L2 (T-cell factor 7-like 2). The risk alleles have been associated with reduced glucose and GLP-1 (glucagon-like peptide 1)-stimulated insulin secretion. Recent evidence has suggested that inheritance of the at-risk T allele at SNP rs7903146 may increase the expression of TCF7L2 in adult human islets. However, the cellular mechanisms by which changes in TCF7L2 levels may affect insulin secretion are unclear. In the present paper, we describe the use of RNA silencing to investigate the role of TCF7L2 on insulin secretion and gene expression in rodent islets. We find that reduced TCF7L2 expression reduces glucose-stimulated insulin secretion and insulin gene expression while slightly potentiating glucose-stimulated changes in intracellular free Ca2+ concentrations.

Genetics of TCF7L2 and diabetes
TCF7L2 (T-cell factor 7-like 2, formerly known as TCF4) is a Wnt signalling-associated transcription factor in several tissues, including the gut [1] and pancreas [2]. Genetic studies, using both case-controlled and whole-genome association methods [3–6], have identified several non-synonymous polymorphisms in the TCF7L2 gene as conferring increased risk of T2DM (Type 2 diabetes mellitus) in non-obese subjects belonging to many different populations [4,6]. The most strongly associated SNPs (single nucleotide polymorphisms), rs12255372 and, particularly, rs7903146, are associated with severe diabetic phenotypes characterized by reduced insulin secretion [4,7] and β-cell function [8] and loss of the GLP-1 (glucagon-like peptide 1) potentiation of insulin secretion [9]. Non-diabetic carriers of these polymorphisms also had a reduced insulinogenic index [10] with no effect on insulin sensitivity. Furthermore, the presence of the at-risk allele is associated with hyperglycaemia in a general French population and reduced insulin secretion in non-diabetic female subjects [11,12]. Taken together, these observations suggest that TCF7L2 may have a major role in regulating glucose-stimulated insulin secretion. However, the molecular mechanism behind the effects on secretion in β-cells is still unclear. Moreover, many questions remain about the effect that the mutations may have on TCF7L2, whether they affect the expression levels, transcript splicing/size, or the function of the mature protein.

Multiple TCF7L2 transcripts have been identified in mammals [13], and, although both T2DM-associated SNPs are located in putative introns, there is as yet no evidence for a role in the coding or activity of the transcription factor.

Expression of TCF7L2
There is an ongoing debate about the effect of the at-risk allele rs7903146T (C/T) on TCF7L2 expression. An earlier study [14] reported increased levels of TCF7L2 mRNA in islets from T2DM patients compared with controls and healthy individuals carrying the at risk T allele. However, the viability of the preparations used and the inability to control for comparative tissue mass and cell type makes interpretation difficult. Moreover, the correlation between increasing expression level of the mutant TCF7L2 mRNA and worsening diabetic condition of the patients was weak. On the other hand, other studies have found no effect of genotype on [4,15], or a decrease in [6], TCF7L2 expression levels in skeletal muscle and adipocytes. As there are multiple possible transcripts of TCF7L2, one possibility is that a genuine increase in mRNA levels may represent increased levels of transcripts encoding less active isoforms. In addition, following the earlier observation from our laboratory [2] that the obese ZDF (Zucker diabetic fatty) (fa/ fa) rat, an animal model of T2DM, also has increased TCF7L2 expression, a possibility arises that increased TCF7L2 expression may be a consequence, not a cause, of defective insulin secretion.

TCF7L2 knockout
Although the study from Group and co-workers [14] suggested that overexpression of TCF7L2 may be deleterious for β-cell function, the effects of TCF7L2 down-regulation have not been explored. Tcf7l2-null mice die shortly after birth [13] and show a loss of a replication compartment in the gut, but other epithelial tissues, including the endocrine...
Figure 1 | Example illustrating the effect of TCF7L2 expression level on insulin secretion in mouse islets
(A) Western blot showing TCF7L2 expression after 48 h of RNA interference. Scram., scrambled. (B) Insulin secretion.

TCF7L2 and β-catenin
TCF7L2 acts downstream of β-catenin in the Wnt signalling pathway (Figure 2). A potential cause of many chronic disease states involving oxidative damage (see [15] for a review) is an imbalance between different pathways involving β-catenin. One example is the FoxO1 (forkhead box O1) transcription factor pathway, which is known to protect cells against oxidative stress. FoxO1 translocates to the nucleus and binds to β-catenin after phosphorylation by JNK (c-Jun N-terminal kinase). As the availability of β-catenin is a rate-limiting step, increases in the pool of free β-catenin, owing to reduced TCF7L2 expression, may increase the activity of FoxO1. This raises the possibility that FoxO1 may play a role in the mechanism of action of TCF7L2 silencing on insulin secretion. However, concomitant silencing of FoxO1 failed to rescue the effect of TCF7L2 knockdown on glucose-stimulated insulin secretion in mouse islets. It remains possible, however, that interactions between the TCF7L2 and FoxO1 pathways may be involved in the age- or stress-related loss of β-cell mass in T2DM. We will use the tools developed to study TCF7L2 expression to investigate this possibility further.

These results demonstrate that TCF7L2 is required for the normal regulation of insulin secretion from mature β-cells by glucose. On-going studies from this laboratory suggest that TCF7L2 is also important for β-cell proliferation (G. da Silva Xavier, M.K. Loder, A. McDonald and G.A. Rutter, unpublished work). Whether decreased TCF7L2 expression is observed during development in individuals with the at-risk allele remains to be explored.

References
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Supported by Wellcome Programme Grants 081958/L/07/Z, 067081/Z/02/Z to G.A.R, and JDRF (Juvenile Diabetes Research Foundation) Postdoctoral Fellowship 3-2005-112 to G. da S.X.


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Received 18 December 2007
doi:10.1042/BST0360357