Review article

To unravel the role of TCF7L2 (Transcription Factor 7 like 2) variants in type 2 diabetes mellitus

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ABSTRACT

Predisposition to diabetes is attributed to a large number of genes. TCF7L2 is one of the most significant candidate genes among them, and it is crucial for beta-cell activity and blood glucose regulation. According to previous research, TCF7L2 has been implicated in T2DM in various Indian tribes. Many studies have discovered a link between TCF7L2 gene variations and vulnerability to T2DM**.** These results suggest that changes in TCF7L2 expression are not only associated with insulin resistance but also with risk for genotypes connected to poor beta cell activity. TCF7L2 drives the conventional Wnt signalling pathway and functions as a nuclear ligand for beta catenin. Wnt signalling is necessary for intestinal endocrine L-cells to emit GLP-1 (glucagon-like peptide-1). As a result, a blockage in this route would cause less GLP-1 to be released, which might have an impact on the release of insulin after meals contributing to T2DM as a result. The molecular processes through which the TCF7L2 gene increases the susceptibility of T2DM will therefore be of significant interest, as will the identification of possible therapeutic targets that may be employed to treat and prevent the illness as a result of the gene variations.

Keywords: TCF7L2; T2DM; Wnt; SNP; GLP-1.

INTRODUCTION

Diabetes mellitus is a category of metabolic illnesses marked by high blood glucose levels. The pathogenic mechanism that leads to hyperglycemia is currently used to classify diabetes mellitus. Type 1 diabetes mellitus is characterised by a shortfall of insulin and a propensity for ketosis, while a diverse set of conditions known as T2DM are characterised by insulin resistance, attenuated insulin production, and increased hepatic glucose synthesis (1). India with '31.7 million in 2000; 79.4 million in 2030', China with "20.8 million in 2000; 42.3 million in 2030", and the United States "17.7 million in 2000; 30.3 million in 2030" has the highest number of diabetics. T2DM has clearly become an outbreak in the twenty-first era, with India leading the globe in the number of diabetics (2).

It is a chronic disorder with long-term effects that includes problems in vision, renal disease, neural ailments, and cardiovascular disease. It is becoming an outbreak with an increased frequency across the globe. Both genetic and epigenetic factors affect how this complex genetic disorder manifests. Due to its population's innate propensity for the disease and rapid population growth, India is amongst the top nations with the highest proportion of diabetics (62.4 million). By the years 2030, this number is projected to reach 100 million due to the alarming rate at which it is growing (3).

Predisposition to diabetes is attributed to the large number of genes. TCF7L2 is one of the most significant candidate genes among them, and it is crucial for β-cell activity and blood glucose regulation.

A transcription factor called TCF7L2 that has previously been linked to blood sugar management is engaged in Wnt signalling. On chromosome 10q25.36, it covers a distance of 215.9 kb (4). It was discovered that a modification in the activity of the TCF7L2 gene, which is most likely brought on by an issue with the CGC gene, which produces glucagon, impairs the pancreatic islets' capacity to function normally. As a result, T2DM risk may increase and secretion of insulin may decrease (5).

TCF7L2

The human TCF7L2 gene, which may be found on chromosome 10q25.3, was first identified in colorectal cancer (CRC) cell lines. With 17 exons, TCF7L2 has a complicated splicing sequence in various tissues. The functional domains of this gene are highly conserved (6). The full-length TCF7L2 protein is shown in Figure 1 which contains two main domains, the N-terminal β-cat binding domain and the HMG-box for DNA binding. Both domains exhibit remarkable interspecies conservation (7).

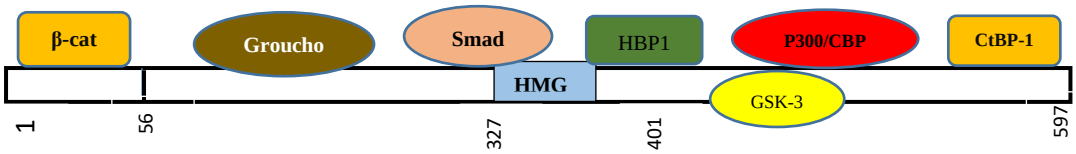

Fig.1: The full-length TCF7L2 protein consists of two major domains including the β-cat binding domain at the N-terminal as well as the HMG-box for binding to DNA. Modified from Ip *et al.*

TCF7L2 also shares the same reliable splicing patterns as the gene for the transcription factor T-cell factor 1 (TCF1), as well as significantly preserved motifs in the 30 region of exon 18. The carboxyl end region of the TCF7L2 isoforms has two binding sequences that have remained constant across time. Because of the extensive TCF7L2 splicing pattern, it is possible that several protein isoforms with oppositional transcriptional activation properties are produced by using different isoforms in the 30 regions.

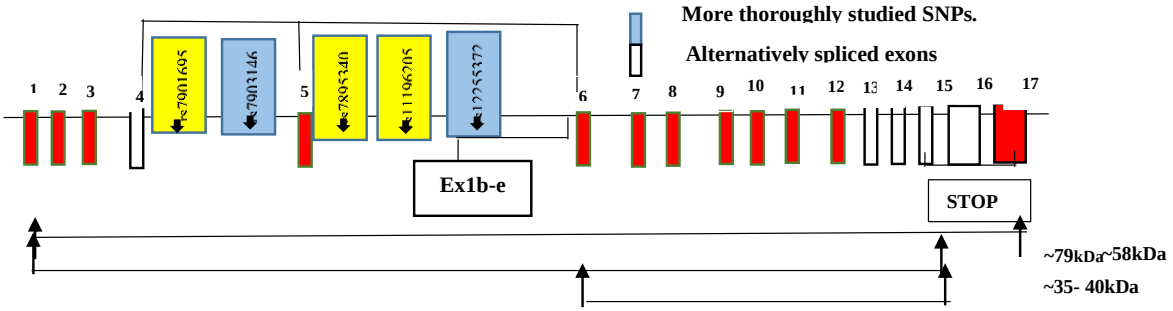

Fig.2: TCF7L2 genetic structure, T2DM risk SNP locations. Modified from Ip *et al.*,

The human TCF7L2 gene, which has 17 exons (boxes) and is found on chromosome 10q25.3, is shown in Figure 2. The alternative splicing involves at least five exons (white boxes). The extensive intronic areas surrounding exon 5 contain five SNPs, all of which were initially found to be related with T2D risk in a variety of ethnic backgrounds The TCF7L2 gene is subjected to a substantial degree of alternative splicing, resulting in a significant number of transcripts and a variety of isoforms. Alternative stop codons produce the size 79 and 58 kDa main isoforms. Upstream of exon 6, an unique transcription start point known as Ex1b-e was recently discovered, which results in the creation of a prevalent TCF7L2 isoform with a mass of 35–40 kDa (7).

TCF7L2 performs its functions through WNT Signalling Pathway

Various initial studies have talked about the function of particular elements of the Wnt signalling pathway in pancreatic β-cell duplication or propagation, in context of typical cholesterol digestion and insulin release induced by glucose, as well as in the development and action of incretin (8).

Even more elaborate investigations started on the involvement of Wnt signalling pathway in glucose breakdown after a study by Grant *et al.* in 2006 (9). This research unveiled the relation of TCF7L2 with the susceptibility of T2DM and its SNPs. In various follow up investigations, it has been repeatedly seen that TCF7L2 SNPs and T2DM risk have a deep rooted relationship.

Canonical Wnt Signalling Pathway

In 1982, Nusse and Varmus conducted research on breast cancer and discovered the earliest Wnt ligand, the INT1 gene. This proto-oncogene was afterwards called WNT1 because there is very much similarity between the sequence of amino acid and the protein encoded in Drosophila (fruit fly) by Wingless (wg), a gene that is important for this insect's segment polarity. 19 ligand-encoding genes of Wnt have been explored in humans and rodents (10).

Frizzled proteins (FRZ) are the name of the Wnt ligands that can be used as receptors. A transmembrane protein called Arrow in Drosophila, which is absolutely necessary for hereditary Wingless signalling, communicates with Wnt ligands. The mammalian homologues of Arrow protein are Low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) and they serve as major coreceptors of Wnt ligands in mammalian proteins (11).

Upon binding of Wnt ligand attached to a frizzled receptor, the canonical (β-catenin dependent) or non-canonical (β-cat-independent) Wnt signalling pathways are triggered (12). The bipartite transcription factor, generated by free β-cat and one of the TCF family members (TCF7L2, TCF7, TCF7L1, and LEF-1), is the primary effector of the Wnt signalling pathway.

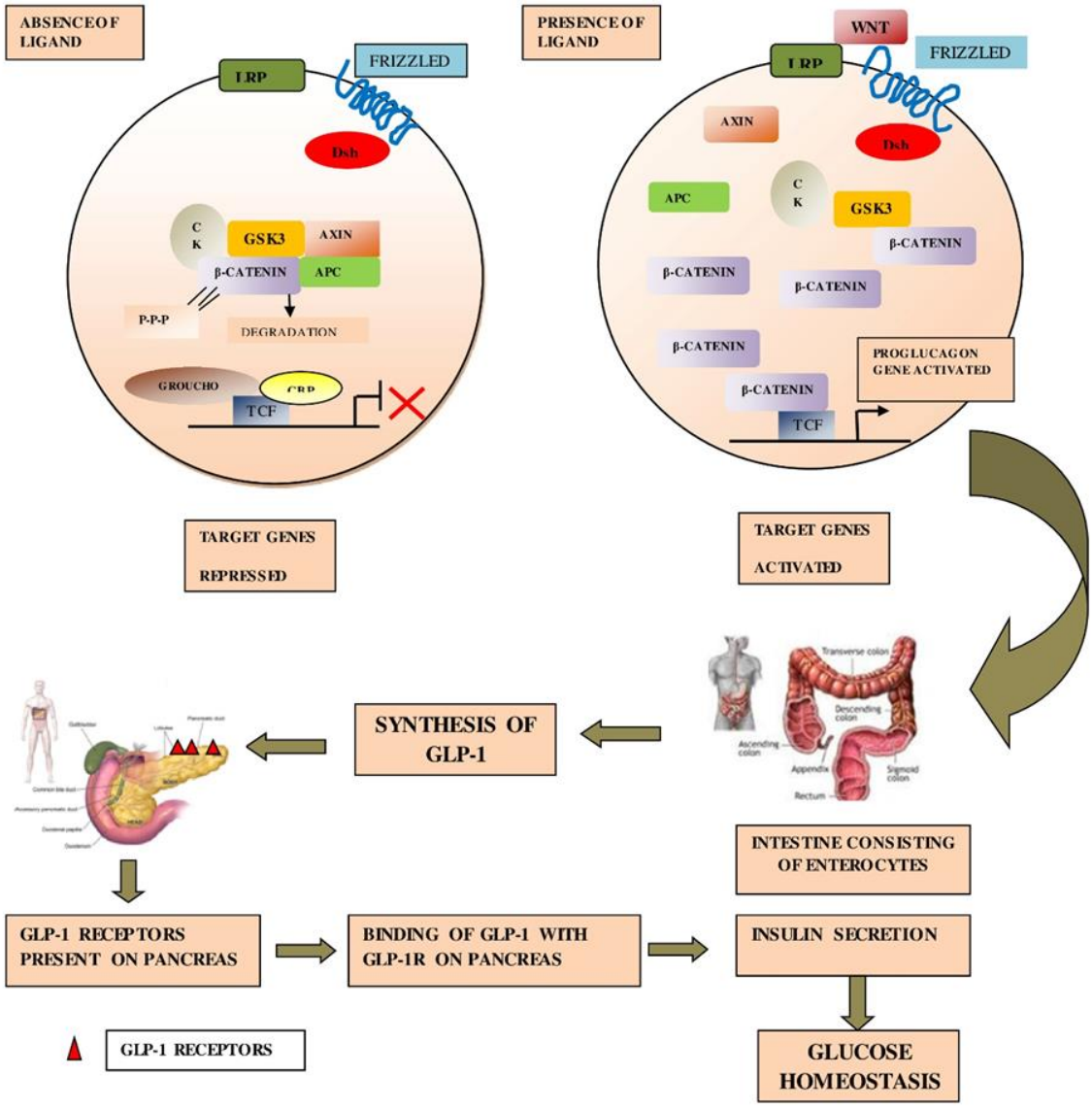

Figure 3A: In the absence of Wnt signal, the transcriptional coactivator β-catenin is not allowed to accumulate and is degraded by a multiprotein "destruction complex". The complex of proteins in destruction complex are tumor suppressors Axin and adenomatous polyposis coli (APC), the Ser / Thr Glycogen synthase Kinases (GSK-3β), Casein Kinases 1 (CK1), Protein phosphatase 2A (PP2A) and the E3 - ubiquitin ligase (β-TrCP / β-transducin repeat containing protein). In the destruction complex, phosphorylation of CK1 at ser45 followed by phosphorylation of Ser33, Ser37 and Thr41 by Glycogen synthase Kinase (GSK3). This phosphorylation generates a β- TrCP recognition site near the β catenin amino terminus, which requires scaffolding of the β catenin and kinases by Axin. Hence, β TrCP mediated ubiquitinated β Catenin is subsequently degraded by the proteasome.
Figure 3B: In the presence of Wnt signal, there is immediate recruitment of Axin protein complex to the activated Wnt receptor followed by GSK-3 beta and CK 1 phosphorylating LRP-5/6 coreceptor. The Axin-1 complex translocation disrupts the process of beta- catenin phosphorylation and degradation. Thus the binding of Wnt ligands to the cysteine – rich domain of Frizzled receptors resulted in disassembly of the destruction complex containing axin, APC and GSK3 leading to stabilization of β catenin. Beta catenin gets accumulated and is eventually imported into the nucleus where it serves as transcription activator of the TCF/LEF-1 family of DNA binding proteins.
This leads to activation of proglucagon gene in enterocytes which synthesizGLP-1. This GLP-1 binds to GLP-1 receptors present on pancreas and cause insulin secretion thus helping in glucose homeostasis.

TCFs have a DNA binding domain of a high mobility group (HMG) box, while β -cat offers domains of transcriptional activation. The proteasome-mediated degradation mechanism regulates the free β-cat level tightly in resting cells. Two tumor suppressors which are members of the β-cat "destructive complex," namely adenomatous polyposis coli (APC) and Axin/conductin, and the serine/threonine kinases glycogen synthase kinase-3 (GSK-3) and casein kinase 1α (CK-1α) are involved in this activity as shown in figure 3A and 3B (13).

The purpose of Axin/conductin and APC is to serve as frameworks, while GSK-3 and CK-1α are involved in phosphorylation of a few serine or threonine residues, including residue Ser33, at the N end of β-cat. β-cat phosphorylated at these build ups at that point goes through proteasome-interceded debasement.

Since Ser33 phosphorylation is essential for proteasome degradation, the β-cat mutation Ser33Tyr (S33Y) produces a persistently functional protein that is frequently employed in studies of the Wnt signalling pathway (14). A connection is framed between the Wnt receptor and Dishevelled (Dvl) on Wnt ligand incitement, which stimulates the separation of the "destructive complex" of β-cat. Free β-cat at that point amasses and arrives at the core, prompting the arrangement of β-cat/TCF and the stimulation of Wnt signalling (or β-cat/TCF) downstream target genes.

In the absence of β -cat, TCFs suppress the transcription of Wnt target genes by enrolling a few nuclear corepressors. TCFs also exhibit transcriptional repressive functions which were demonstrated by initial research in Drosophila, Xenopus, and *Caenorhabditis elegans* (roundworms; 15).

Comparable results were seen by investigations on the Siamois enhancer in frogs (16). While nuclear β -cat aggregates were abundant in the dorsal regions where this enhancer was active, the ventral counterparts had relatively little activity. In this way, for TCF to work as a transcriptional activator, it has to rely on the accessibility of β-cat.

The capacity of TCFs to recruit transcriptional repressors was then revealed by mechanistic explorations. In Xenopus, the first repressor has been reported. Roose *et al*. showed a physical relationship among XTCF3 and transcriptional repressor individuals from the Groucho family.

C-terminal restricting protein (CtBP)- 1 is another very much archived transcriptional repressor that intervenes the suppressive action of TCFs. To apply its oppressive impact on Wnt-interceded gene expression, Frog XCtBP appeared to collaborate legitimately with the carboxyl end of XTcf-3. By selecting histone-altering enzymes that include suppressive histone marks and remove actuating marks, CtBP proteins automatically do as such. CtBP proteins may also be self-associated with gene regulatory complexes and possibly carry them together (17).

Cauchi *et al.*, reported diminished TCF7L2 expression in subcutaneous and omental fat tissue in T2DM patients (18), while Kaminska *et al.*, revealed that weight reduction may control the excess of spliced types of TCF7L2 in fat tissue (19).

In addition, Liu and Habener also showed that the glucagon-like peptide-1 (GLP-1) has a stimulatory effect on phosphorylation of β-cat Ser675. β- cat acetylation is another modification that controls the activity of β-cat / TCF and is seen post-translationally (20).

Groves *et al.*, performed an analysis and showed a significant relationship between SNPs rs7903146 and rs4506565 with likelihood of developing T2DM (21).

Wang *et al.*, confirmed the link between the prevalent Variant rs12255372 [T/G] and the likelihood of developing T2DM in Caucasians (22).

Attempts were undertaken to look into the underlying mechanisms as soon as it was discovered that there was a link between T2DM risk and TCF7L2 mutations. It has been established that Wnt signalling regulates both the incretin hormone GLP-1's development and the expression through the gut proglucagon gene (23).

The intronic regions of this gene presently contain all known T2DM risk SNPs. It is clear that these SNPs have an impact on TCF7L2 activity because they do not alter the coding sequence. Differential adipose tissue splicing and TCF7L2 expression have both been linked to risk SNPs, and attempts have been undertaken to analyse this relationship (24).

GLP-1 The primary homeostasis determinant of blood glucose

GLP-1, a peptide released in response to a meal by intestinal enteroendocrine L cells, affects blood glucose balance in people. L cells in reaction to luminal carbohydrates, amino acids, and fatty acids, they serve as nutrient sensors to release GLP-1. Released GLP-1 slows gastric emptying inside the intestinal wall by activating enteroenteric reflexes necessary for controlling the gastric mucosa.

GLP-1 released at the same time stimulates vagal sensory nerve terminals that supply the intestinal wall, and thus starts vagal-vagal autonomic reflexes that direct the movement of the endocrine pancreas. In the islets of Langerhans in the endocrine pancreas, GLP-1in circulation also serves as a hormone to induce the release of insulin, whereas suppress the release of glucagon. These immediate and numerous GLP-1 activities function in conjunction to lower blood glucose levels during the postprandial process of blood glucose regulation (25).

Insulin like properties of GLP-1

It is currently acknowledged that the dominating bioactive GLP-1 present in human serum is GLP-1 (7–36) amide. It has a MW of 3298 Da and is made out of 30-amino-acid residues with the arrangement HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRN2. This condensed, completely bioactive GLP-1 increases the release of insulin induced by glucose by attaching to the human GLP-1R expressed on islet cells. Importantly, GLP-1's insulin secretagogue activity is followed by its ability to induce transcription of insulin genes, translation of insulin mRNA, and biosynthesis of proinsulin in β-cells (26).

GLP-1 likewise works as a β-cell development factor, potentially just as significant, with the goal that it advances β-cell expansion in mice while additionally applying an antiapoptotic activity to secure from β-cell death (27). Therefore, it is of great interest to determine if such preclinical results are relevant to GLP-1R agonists treated T2DM patients in relation to GLP-1.

GLP-1R is a G protein-coupled receptor (GPCR) that is among other seven transmembrane-spanning domain proteins of the secretin receptor-like family. GPCRs that preferentially engage secretin, glucagon, glucose-dependent insulinotropic peptide (GIP), vasoactive intestinal peptide (VIP), and pituitary cyclase-activating peptide adenylyl (PACAP) are included in these group B receptors. In light of agonist binding to GLP-1R, heterotrimeric GS GTP-binding proteins are initiated and interface GLP-1R agonist inhabitancy to transmembrane adenylyl cyclase (TMAC) incitement (28).

TMACs catalyse ATP to cytosolic cAMP conversion in β-cells. Either Protein kinase A (PKA) or Epac2-

assigned cAMP-directed guanine nucleotide exchange factor are activated by the signalling molecule cAMP. The function of PKA is to phosphorylate the β-cell stimulus-secretion linkage and gene controlling networks of main substrate proteins. On the other hand, through Rap1 GTPase, Epac2 initiates a new phospholipase C-epsilon (PLCε) which explicitly hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) (29).

β-cell gene expression is mediated by a protein kinase A mediated activity of GLP-1R agonists that phosphorylate CREB, a cAMP response element-binding protein. Protein Kinase A activation is combined with stimulation of gene transcription by binding of cAMP response elements (CREs) situated in gene promoters of 5' through activated CREB. GLP-1R agonists in β-cells influence several CREB regulated genes (30).

It is interesting to find that, Protein Kinase A-interceded phosphorylation of β-catenin likewise brings about a proliferative activity of GLP-1, recommending that the β-cell cAMP-Protein Kinase A signalling branch shows crosstalk signal transduction with a non-canonical Wnt signalling pathway that utilizes the TCF7L2 to modulate gene expression.

The affinity for ligand binding and selectivity are dictated by collaborations between the GLP-1(7-36) amide C end and the N-terminal area of the receptor, while the receptor centre space and its intracellular loops are strongly influenced by the combining of the GLP-1R to intracellular signalling pathways. After GLP-1(7-36) amide binds to GLP-1R, the peptide undergoes a fundamental conformational alteration to activate receptor signalling (31).

CONCLUSION

According to the research mentioned above, there is a strong correlation between polymorphisms in the TCF7L2 gene (formerly known as TCF4) and the likelihood that someone may have T2DM. By regulating CGC expression and GLP-1 levels in plasma, the variants of TCF7L2 may cause T2DM. As a result of its anticipated therapeutic potency in T2DM, GLP-1 has received a lot of interest.

According to a study, β-catenin is essential for controlling insulin secretion, and overexpressing beta-catenin transcriptional co-activator, TCF7L2, reduces insulin secretion. Unknown mechanisms underlie how beta-catenin overexpression affects insulin secretion. Clarifying it might result in a whole new method of treating T2DM.

Clinical significance

India has the most diabetic subjects of any single nation, with a prevalence of "12% - 16%" in urban areas. Studies on the genetics of T2DM in the Indian population are few and far between. Variations in the TCF7L2 gene have been strongly attributed to the probability of occurrence of T2DM.

The molecular processes by which the TCF7L2 gene contributes to the etiology of type 2 diabetes will therefore be of significant value, as will the identification of possible therapeutic targets that may be utilized to treat and prevent the illness as a result of the gene variations. It is alluring to imagine a genetic test in the future that may detect people who carry the at-risk TCF7L2 gene variations and provide them with preventive healthcare. A thorough comprehension of the mechanism will aid in the creation of possible novel treatments for T2DM.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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