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Research article

Identification of dysregulated pathways through *SLC30A8* protein interaction in type 1 diabetes mellitus

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Abstract: *Objective:* The aim of the current study was to explore the gene enrichment and dysregulated pathways on the basis of interaction network analysis of *SLC30A8* in type 1 diabetes mellitus (T1DM). *SLC30A8* polymorphism could be characterized as a beneficial tool to identify the interacting gene in developing T1DM. *Materials and methods: SLC30A8* interacting protein interaction network was obtained by String Interaction network Version 11.0. Ten proteins were identified interacting with *SLC30A8* and were analysed by protein-protein interaction and enrichment network analysis along with Functional Enrichment analysis tool (FunRich 3.1.3) to map the gene data sets. In entire analysis, FunRich database was used as background against all annotated gene/protein list. Protein-protein interaction (PPI) and enrichment network analysis of the selected protein: *SLC30A8* gene along with gene mapping and pathway enrichment were performed using FunRich 3.1.3 and String Interaction network Version 11.0. *Results:* Biological pathway grouping displayed enriched proteins in TRAIL signalling pathway ($p < 0.001$). *PTPRN, GAD2* and *TCF7L2* were enriched in TRAIL Signalling pathway when *INS* was made focused gene and directly interacting with *SLC30A8*. *Conclusions*: TRAIL signalling pathways were enriched in T1DM. Therefore, *SLC30A8* along with *PTPRN, GAD2* and *TCF7L2* involved in TRAIL pathway must be further explored to understand their in vivo role in T1DM.

Keywords: TRAIL; SLC30A8; diabetes mellitus; type 1; pathways; protein interaction

1. Introduction

Type 1 diabetes mellitus (T1DM) is an immune mediated destruction of pancreatic β cells that targets autoantigens showing high expression of β-cell specificity [1]. It is considered as a childhood disease but can be diagnosed in later years of life as well [2]. The incidence of T1DM in Pakistan is 1.02 per 100,000 every year [3]. Genetic agents and environmental factors contribute in the rate of incidence and variable clinical features of T1DM among different populations [4]. It is affirmed by the fact that there is an increased risk of T1DM in individuals who have a first degree relative with diabetes [5].

The conventionally used antibodies for the diagnosis of T1DM are against insulin (INS), Glutamic acid decarboxylase (GAD) and tyrosine phosphatase (IA-2) were unable to diagnose all the patients, so some newer antibodies has been identified as a major diagnostic marker for T1DM against Zinc transporter 8 to expand the panel of diagnostic autoantibodies [6]. ZnT8 is encoded by *SLC30A8* gene that is located on the q arm of 8^{th} chromosome at 23.11 position. It contains 369 amino acids [7].

Despite of new discoveries, its diagnosis is very difficult before the clinical manifestation of the disease. The differentially expressed genes (DEG) have been detected by many researchers between T1DM and normal individuals but different databases have showed variable results for differentially expressed genes (DEG) [8,9]. To clarify the exact physiology and the cellular processes, pathway-based analysis has been suggested [10]. Based on gene expression levels, several methods have been developed for the detection of possible pathways involved in the disease for e.g., Generally applicable gene-sets enrichment, Over-representation Analysis and Gene Set Enrichment Analysis [9,11]. Gene interactions play a crucial role in the pathways as their change have a significant impact on various activities of the pathways [12]. A topology based approach was developed, EnrichNet for studying the dysregulated pathways in previous years but it was not significant [13].

The evolution of bioinformatics has been proved to be beneficial in new drug designing, in developing new biomarkers and in the planning of different treatment strategies. In reference to the existing literature, the aim of this study was to figure out the gene enrichment of *SLC30A8* and dysregulated pathways based on interaction network analysis of *SLC30A8* in T1DM.

2. Materials and methods

2.1. Gene association data

This is an in-silico study, the idea of *SLC30A8* was acquired by molecular study in an earlier study conducted by some authors of the study that was approved by ERC (Ethics Review Committee) of Ziauddin University [14]. *SLC30A8* gene interaction pathways and gene expression were done in this in silico analysis. Molecular analysis of *SLC30A8* revealed significant association of this gene polymorphism with ZnT8A in the former study concluding negative association of *SLC30A8* polymorphism with type 1 diabetes mellitus (T1DM). Hence, *SLC30A8* polymorphism could be characterized as a beneficial tool to rule out the appearance of ZnT8A for early diagnosis of T1DM.

2.2. Bioinformatic analysis

SLC30A8 interacting protein interaction network was obtained by String Interaction network Version 11.0 [15]. The STRING database aims to integrate all known and predicted associations between proteins, including both physical interactions as well as functional associations.

Identified interacting protein (10) with *SLC30A8* were analysed by protein-protein interaction and enrichment network analysis along with Functional Enrichment analysis tool (FunRich 3.1.3) [16] to map the gene data sets and interaction pathways.

2.3. Enrichment analysis

Enrichment analysis was performed for molecular functions, biological pathways, gene ontology terms and site of expression terms. The depleted and enriched proteins were recognized by calculation of the fold change for protein domains, biological pathways and site of expression.

2.4. Interaction network analysis

The PPI network visualization and analysis were executed using biological pathway enrichment of defined nodes. Only human specific datasets were hosted in which gene/protein annotations were gathered from publicly accessible protein-centric and gene databases. The background database used in complete analysis was exclusively human specific FunRich database. It provided more than 1.5 million annotations relating to pathways, disease associations, sites of expression (normal and disease tissues, cell lines), protein domains, transcription factors, molecular function, cellular component, protein-protein interactions and biological process. Diverse interaction network arrangements like planetary, circular, packed and square were applied in the analysis. List of genes enriched in specific pathways was emphasized within the interactions network and distinct sub-network were produced for detailed analysis. Particular sub-network was analysed by adding direct neighbours (interacting partners) for those nodes in the sub-network and envision. Particular nodes (INS) were focused as its involvement in the pathogenesis of T1DM is experimentally proven, and the interacting partners of the focused nodes were plotted.

BH and Bonferroni tests were applied in FunRich Software hypergeometric test. Using the p-value correction with the BH and Bonferroni tests and hypergeometric test, gene ontology (GO) functional categories, normal and Overrepresented and identified pathway associations and significant interactions with datasets. The statistical cut off p-value < 0.05 of enrichment analysis was maintained as default after Bonferroni correction. In entire analysis, FunRich database was used as background against all annotated gene/protein list.

3. Results

3.1. Interacting proteins for SLC30A8 gene

Ten proteins were identified that were directly interacting with SLC30A8: *GAD2, SLC30A6, G6PC2, INS, PTPRN, HHEX, KCNJ11, TCF7L2, IGF2BP2 and CDKAL1* (Figure 1).

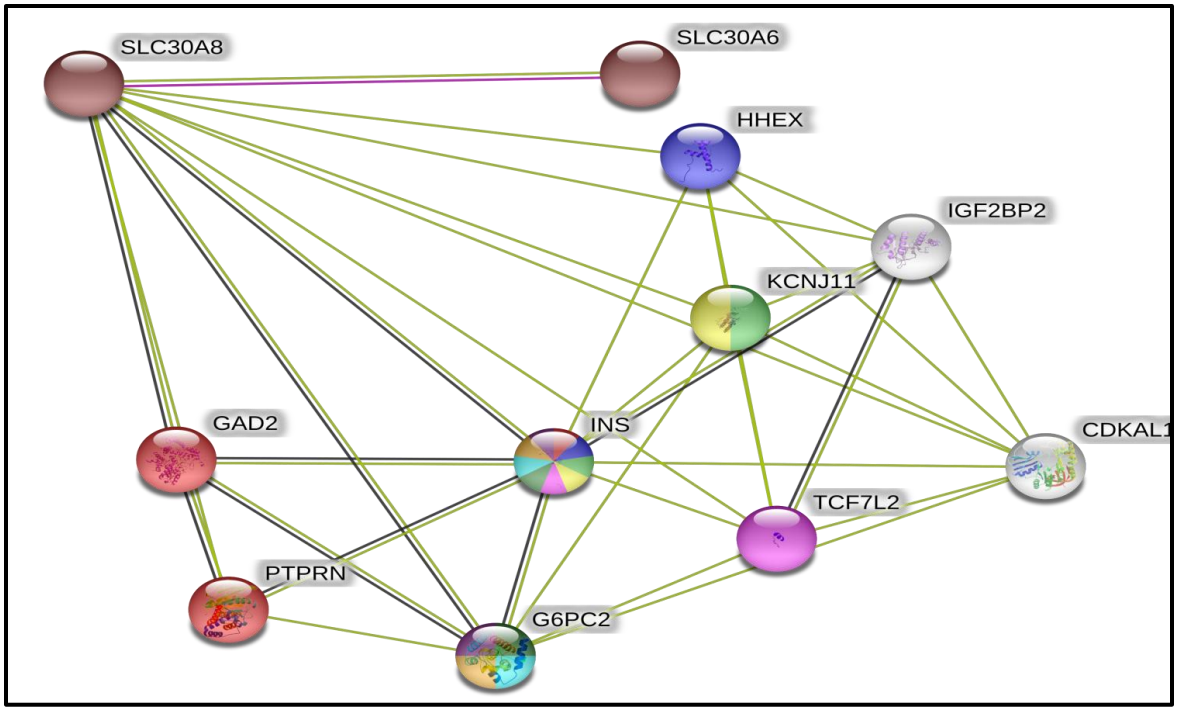

Figure 1. String image of *SLC30A8* interacting proteins. Green line: gene neighbourhood; black: co-expression; and purple: experimentally determined.

3.2. Protein-protein interaction (PPI) analysis of SLC30A8

Using FunRich database, the PPI network was viewed and *SLC30A8* have studied. The interaction network was used in the pathway enrichment of the identified protein. From the interaction of *SLC30A8*, differentially regulated interacting proteins were potentially recovered as shown in Figure 1. There were 10 proteins discovered to interact with *SLC30A8* gene, some of which interacts with each other as shown in Figure 1. Table 1 enlists the genes interacting with *SLC30A8* gene along with their proteins, mapped location on chromosomes and enriched pathway. CDK5 regulatory subunit associated protein 1-like 1, Insulin, insulin-like growth factor 2 mRNA binding protein 2 and Transcription factor 7-like 2 (T-cell specific, HMG-box) were among the proteins mapped. TRAIL signalling pathway, Insulin Pathway, Proteoglycan syndecan-mediated signalling events, Glypican pathway, ATP sensitive Potassium channels, Integrin-linked kinase signalling, Sphingosine 1-phosphate (S1P) pathway, S1P1 pathway,

AP-1 transcription factor network, EGF receptor (ErbB1) signalling pathway and Arf6 downstream pathway were the major and linked pathways found interacting with their proteins as shown in Table 1, displaying the genes interacting with *SLC30A8* in the following pathways.

Table 1. Gene mapping and biological pathways enriched in *SLC30A8* protein interaction.

Gene symbol	Protein Name	Chromosome	Map location	Interacting Genes with SLC30A8	Biological Pathway
SLC30A8	solute carrier family 30 (zinc transporter), member 8	8	8q24.11	TCF7L2; INS; EP300; INSR; MYC; PIN1	TRAIL signalling pathway
SLC30A6	solute carrier family 30 (zinc transporter), member 6	2	2p22.3	TCF7L2; INS; EP300; INSR; MYC; PIN1	Proteoglycan syndecan-mediated signalling events
GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)	10	10p11.23	TCF7L2; INS; EP300; MYC; PML; CTNNB1	Integrin-linked kinase signalling
PTPRN	protein tyrosine phosphatase, receptor type, N	2	2q35-q36.1	TCF7L2; INS; EP300; INSR; MYC; PIN1	Sphingosine 1-phosphate (S1P) pathway
G6PC2	glucose-6-phosphatase, catalytic, 2	2	2q24.3	TCF7L2; INS; EP300; INSR; MYC; PIN1	Glypican pathway
INS	insulin	11	11p15.5	TCF7L2; INS; EP300; MYC; PML; CTNNB1	AP-1 transcription factor network
KCNJ11	potassium channel, inwardly rectifying subfamily J, member 11	11	11p15.1	KCNJ11; KCNJ8; ABCC9; ABCC8	ATP sensitive Potassium channels
HHEX	hematopoietically expressed homeobox	10	10q23.33	TCF7L2; INS; EP300; INSR; MYC; PIN1	S1P1 pathway
IGF2BP2	insulin-like growth factor 2 mRNA binding protein 2	3	3q27.2	TCF7L2; INS; EP300; INSR; MYC; PIN1	Arf6 downstream pathway
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1	6	6p22.3	TCF7L2; INS; EP300; INSR; MYC; PIN1	Insulin Pathway
TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box	10	10q25.3	TCF7L2; INS; EP300; INSR; MYC; PIN1	EGF receptor (ErbB1) signalling pathway

Notes: * p-value of all the proteins were significant, i.e., < 0.001.

TCF7L2, EP300, INS, MYC, INSR, GAD2, PTPRN and PIN1 were among the 33 proteins enriched in TRAIL Signalling Pathway with $p < 0.001$. Among all the genes, *INS* was selected as the target gene and directly interacting with *SLC30A8*. With more than 2-fold protein enrichment, GAD2, PTPRN, and TCF7L2 were found to be enriched in the TRAIL Signalling pathway, as shown in Figure 2.

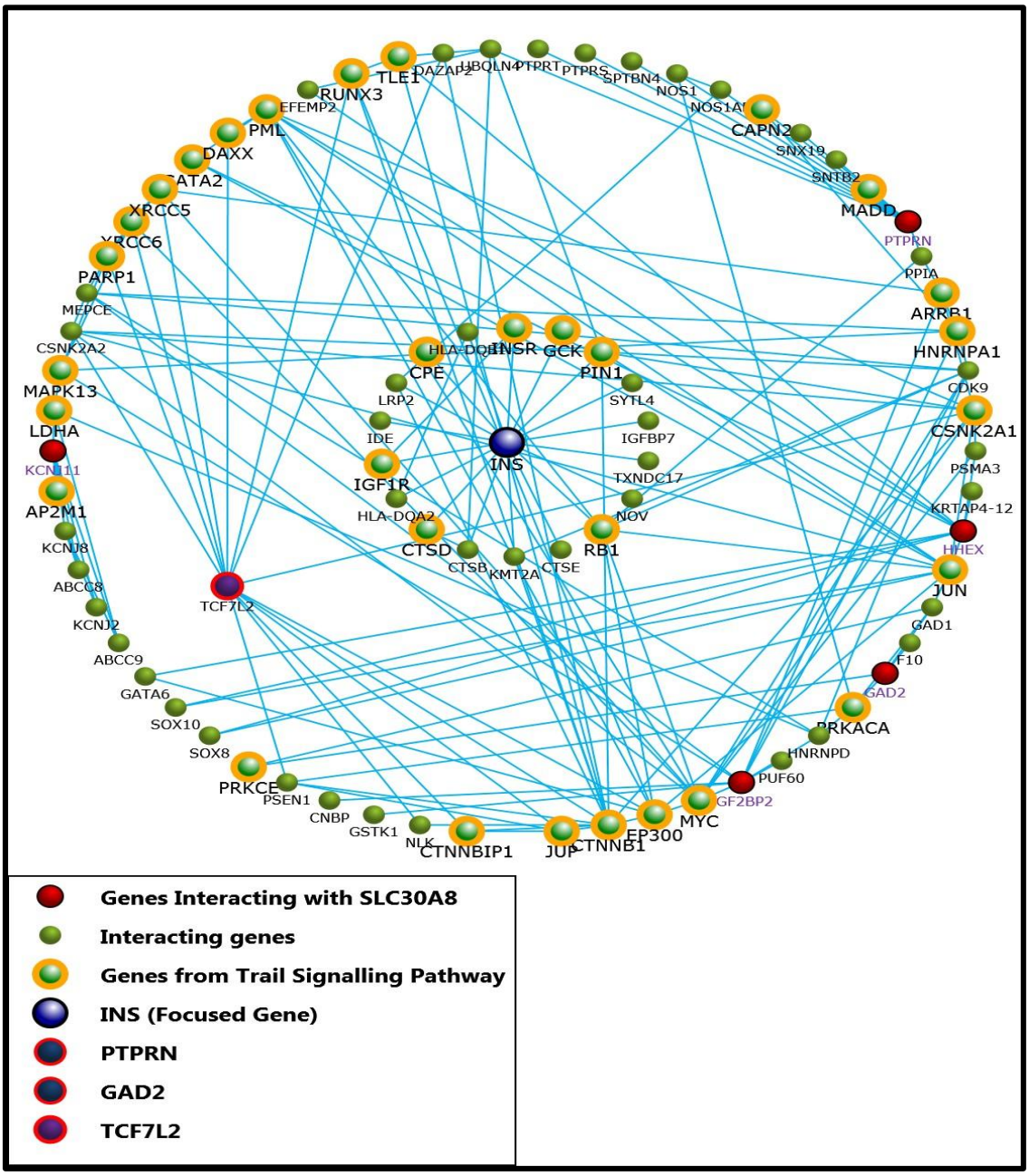

Figure 2. Protein-protein interaction network of *SLC30A8* interacting genes when INS was selected as the focused gene in TRAIL signalling pathway.

4. Discussion

Type 1 diabetes mellitus is an immune-mediated destruction of pancreatic β-cells that produces insulin. It is a complex disease involving ill-defined external insults in the presence of genetic susceptibility of pancreatic β-cells under the influence of abnormal immune responses [17]. Alteration in zinc homeostasis has a significant impact on the host defence and the immune response [18]. The transport of Zn^{2+} ions are facilitated by the ZnT8 to the insulin vesicles and is also involved in the storage, structural stabilization, secretion of insulin and its action. *SLC30A8* gene encodes for Zinc transporter 8 (ZnT8). Zinc Transporter 8 autoantibodies (ZnT8A) has found to be significantly associated with T1DM [14]. Diagnosing T1DM early is a persistent challenge so the identification of gene and their interacting pathways are crucial in early diagnosis of the disease and also aid in planning the target modalities accordingly.

For better understanding of cellular pathways, biological mechanisms and disease states pathway analysis are believed to be significant. Dysregulated pathways may aid in the detection of better biomarkers compared to single gene. The microarray data gathered till now allowed for the successful recognition of dysregulated pathways [9]. Utilization of network-based strategies have gained concern to retrieve genes and pathways involved in the pathogenesis of complex diseases like T1DM [19]. For example, in the current research, a novel approach based on a gene co-expression network was developed to categorize pathways linked to T1DM. Several novel and advanced methods have been developed to aid in the detection of dysregulated pathways in a variety of diseases. The detection of dysregulated pathways involved in the development of T1DM was done using a network-based approach. In the current research, the dysregulated pathways were identified using a combination of a target network and pathway network. In addition, a network model was used to assess the importance in this method. This study may provide new insight about the disease's biological basis. Recent literature suggests that C/T polymorphism of *SLC30A8* may contribute in the development of T1DM and individuals at risk can be recognized before the development of the disease.

PPI networks were used to identify the disease specific networks based on close protein interactions. *INS, TCF7L2, INSR, PINI, PTPRN, EP300, GAD2* and *MYC* were among the 33 proteins that were found enriched in TRAIL Signalling Pathway with $p < 0.001$. This conclusion is likely by mediating autoimmunity in T1DM and by assisting in crosstalk between TRAIL signalling pathways and the *SLC30A8* gene. This TRAIL pathway is thought to play a vital role not only in autoimmunity, but also mediating the inflammatory processes and programmed cell death of pancreatic β cells. Involvement of only TRAIL pathway in T1DM has been identified in a number of studies but it was discovered that biological pathways associated with *SLC30A8* were signalled by the TRAIL pathway, which had not previously been identified or recognised earlier [20–22].

TNF Related Apoptosis Inducing Ligand (TRAIL) is a well-known signalling pathway of TNF family members that cause apoptosis and are involved in T1DM development [20]. Overexpression of TRAIL induced by augmenting TIMP-1 (Tissue Inhibitor of Metalloproteinase-1) can provide novel therapeutic targets and aid in the prevention of T1DM. It can cause the inhibition of MMPs (Matrix

Metallo Proteinases) activity by the downregulation of the transmigrating diabetogenic T-cells into islets of pancreas finally providing protection to the β cells [21,23]. Moreover, when *INS* was made the target gene, other interesting genes such as *TCF7L2*, *GAD2* and *PTPRN* were enriched in the TRAIL Signalling pathway and found directly interacting with *SLC30A8* with more than 2-fold enrichment. Higher C-peptide levels are linked to the TCF7L2 genetic variation, which may play a role in the development of T1DM. PTPRN is found on the membranes of secretory granules [24,25].

It is proposed that TRAIL is mostly expressed in peripheral islet cells, making them resistant to apoptosis [26]. Beneficial and novel targets for clinical action and prevention of T1DM can be developed by exogenously controlling the expression of the TRAIL pathway. As a result, *SLC30A8*, as well as *PTPRN*, *GAD2* and *TCF7L2*, which are all active in the TRAIL pathway, need to be studied further in order to understand their in vivo function in T1DM.

5. Conclusions

In this analysis, an important pathway and its interacting genes were recognized on the basis of protein-protein interaction of network. Through protein interaction studies, TRAIL signalling pathways were found enriched in T1DM. *PTPRN*, *GAD2* and TCF7L2 were enriched in TRAIL signalling pathway. These genes in TRAIL pathway are the potential targets to diagnose and prevent the disease at an earlier stage.

Conflict of interest

The authors declare no conflict of interest.

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