Identification of new key genes for type 1 diabetes through construction and analysis of the protein-protein interaction networks based on blood and pancreatic islet transcriptomes

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Abstract:

Background: Type 1 diabetes (T1D) is an autoimmune disease in which pancreatic beta cells are destroyed by infiltrating immune cells. Bilateral cooperation of pancreatic beta cells and immune cells has been proposed in progression of T1D, but there is no systems study to investigate it. Here, we aimed to explicate etiological molecular basis and identify key genes associated to T1D risk by network biology approach in two circumstances.

Methods: We integrated interactome (protein-protein interaction (PPI)) and transcriptomes data data of transcriptomes to construct networks of differentially expressed genes in peripheral blood mononuclear cells (PBMCs) and pancreatic beta cells. Centrality , modularity and clique analyses of the networks were implemented to get more meaningful biological information.

Results: By analysis of genes expression profiles, we found several cytokines and chemokines in beta cells and their receptors in PBMCs, which is propagation in the dialog between these two tissues within their protein-protein interactions. High connectivity (hub) and high betweenness (bottleneck) nodes were identified by analyzing of PPI networks to be biologically significant nodes. Then, functional modules and complexes were determined to reveal biological pathways. Immune response, apoptosis, spliceosome, proteasome and pathways of protein synthesis were the most significant pathways in the tissues. Finally, YBX1, SRPK1, PSMA1, PSMA3, XRCC6, CBL, SRC, PIK3R1, PLCG1, SHC1 and UBE2N were identified as key markers which were hub-bottleneck nodes involved in functional modules and complexes.

Conclusions: Our results provided a better understanding of T1D pathogenesis as well as new insight into network biomarkers which may be considered as potential therapeutic targets.

Key points

The significant findings of the study: This is the first study which propagated the concept of dialog between pancreatic islets and immune system in T1D via systems biology view. Interactome-transcriptome analysis revealed high centrality genes differentially expressed in PBMCs and pancreatic beta cells in T1D.

This study adds: This study delineated more potential undergoing mechanisms of T1D and identified key markers for more experimental validation by network biology analysis of two involved tissues.

Key words: Protein-protein interaction network; transcriptome; topology; type 1 diabetes

Introduction

Type 1 diabetes (T1D) is an autoimmune disease with a strong genetic component, during which the pancreatic beta cells in the islet of Langerhans are selectively destroyed via activation of cellular immunity against self-antigens on these cells. This may efficiently hamper endogenous insulin production.[1, 2]

Genome-wide association studies (GWAS) data implicates the involvement of classical immunoregulatory pathways such as modulation of the IL-2 pathway, cytokine signaling and changes in subsets of T cells in T1D.[3, 4] However, it becomes conspicuous from the earlier studies that this immunodysregulation results in T1D development, provided that initial beta cells damage has been occurred.[5] In this regard, recent studies showed that pancreatic beta cells themselves express and release many cytokines and chemokines as early T1D might be influenced by this issue.[6, 7] Therefore, it seems gene expression changes both in pancreatic beta cells and in immune effector cells may be needed to elucidate the disease molecular mechanisms. It is not easy to prepare pancreas samples of new-onset diabetic type 1 humans

because the death rate with appropriate management is extremely low.[8] To address this issue, there is evidence that exploration of the full transcriptome of beta cells exposed to proinflammatory cytokines, such as interleukin-1b (IL-1B), tumor necrosis factor- α (TNF-α) and interferon-ɤ (IFN-ɤ), illustrates a snapshot of the responses of these cells under conditions which may dominate in early T1D.[9] This procedure is considered as a well-established *in vitro* model of T1D pathogenesis.[10] Further to this, it has been supposed that islet-infiltrating immune effectors are in equilibrium with circulating pools and may be sampled in peripheral blood mononuclear cells (PBMCs).[11, 12] The earlier studies have demonstrated that transcriptional profiling of PBMCs is a helpful tool for identifying gene expression signatures of autoimmune diseases.[13, 14]

Some biomarkers of human diseases have been successfully identified through genome-wide analysis of gene expression profiles.[15, 16] However, this method has failed in introducing of reproducible individual gene markers in some studies.[17, 18] Furthermore, gene expression measurements for sorting genes into classical pathways or functional categories were not so effective to reveal disease markers because these methods might be limited to prior knowledge. To at least partially address this shortage, network biology and systematic bioinformatics data such as protein-protein interactions (PPI) and related pathways were introduced. [19] The aim of protein-protein interaction studies is to characterize known associations among the proteins in context of biochemistry, signal transduction and biomolecular networks. [20] In the recent years, the integrated analysis of large-scale gene expression data with PPI networks and topological analysis of subnetworks have prepared a promising approach to obtain a meaningful biological context in terms of functional association for differentially expressed genes. [21-23]

In this study, Query-Query PPI (QQPPI) networks were constructed for T1D using genes which have different expression levels in PBMCs and pancreatic beta cells. Bilateral

cooperation between two tissues was explored via systems biology viewpoint. Topological analyses were performed, and functional modules & complexes were characterized in each network; several biological processes and pathways were identified by these analyses. According to association in functional module & complexes and the degree of centrality measures, some new key markers were identified. This is the first study by which significant markers have been introduced for T1D by topological and functional analysis of differentially expressed genes both in immune cells and pancreatic beta cells. The strategy of our work is shown in Figure 1.

Methods

Sources of gene expression data

In case of PBMCs gene expression profile, the raw data (CEL file) of microarray series data GSE9006 were downloaded from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/). GSE9006 was published by Ellen C *et al*. in 2007; [14] blood samples were obtained from 43 newly diagnosed T1D patients and 24 healthy subjects (mean age (9.5 vs 10.9 years) and the female to male ratio (25/18 vs 14/10)). To achieve pancreatic beta cells gene expression profile, we used the RNA-seq analyzed data of human pancreatic islet transcriptomes (GSE35296) that it was prepared by De´ cio L. Eizirik *et al*. in 2012. [7] Human islets were obtained from the donors who were heart-beating organ donors with no medical history of diabetes or metabolic disorders. Five islet samples were isolated and cultured under control conditions and in the presence of cytokies (IL-1B + IFN-ɤ). On average, preparations contained 58% beta cells, which is similar to reported percentage of 54% in isolated human islets and 55% in human pancreas.

Selection of differentially expressed genes

In case of PBMCs, RNA samples were analyzed by Affymetrix Gene Chip A (HG_U133A) and B (HG_U133B). Each chip (HG_U133A, HG_U133B) were normalized separately by

"affy" package of Bioconductor project. [24] After Robust Multi-array Average normalization, where a gene had more than one probe on the microarray, the average expression value of all the related probes was used to estimate expression level of the gene. The differentially expressed genes were computed based on ANOVA, considering additional variables such as ethnicity, race, age and gender effects between diabetic and control samples. The genes of corresponding probes with p-value < 0.05 were determined as abnormally expressed. For each gene, the minimal p-value (between HG_U133A and HG_U133B) was chosen. To estimate the fold changes in one condition vs another, a linear regression was performed. [25] The fold change of each gene was determined by the chip which gave the more significant p-value. In case of pancreatic beta cells gene expression, over and under expressed genes were extracted by Fisher exact test and the p-values were corrected by the Benjamin-Hochberg (p-value < 0.05). [7]

2.3. Construction and topological analyses of the QQPPI networks

The abnormally expressed genes in PBMCs and pancreatic beta cells were separately located on human PPI network which integrated from three major IMEx [26] public databases: IntAct, [27] MINT [28] and DIP [29] to construct Query-Query PPI (QQPPI) networks, i.e., networks consisting of query genes as the nodes and direct interactions among them. Our recent study showed IMEx databases (especially IntAct and DIP databases) have the more number of significant correlations for their proteins' topological features than the all other paired comparisons between BIND, HPRD, MINT, IntAct and DIP databases. [30] The subnetworks of QQPPI were constructed using Cytoscape software 3.2.0. [31] Topological properties of QQPPI networks were analyzed by this software.

The topologically significant nodes were extracted from the networks in two steps: (1) In the networks, nodes with degree greater than or equal to the sum of mean and twice the standard deviation (S.D.), i.e., mean +2*S.D. of the degree distribution, were considered as

hubs. [32] (2) We defined bottlenecks as the proteins that were in the top 5% in terms of betweenness centrality.

Identification and annotation of functional modules and complexes

Clustering with overlap neighborhood expansion (ClusterONE) algorithm was used in order to identify the connected regions within the network with possible overlap. [33] The modules were identified to have a minimum density of > 0.05 and a degree of > 5. A cluster with a *p*-value of < 0.05 was determined to be a module. The functional meaning for identified modules was further explored, and they considered as candidate functional modules if their genes were significantly enriched in biological process of Gene Ontology (GO) annotation or KEGG/REACTOME pathways. For this analysis, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool, [34] with two cut-off criteria: Benjamin-Hochberg corrected *p*-value <0.05 and the number of genes with specific GO terms >2.

Using Clique Percolation Method provided by CFinder software, the involving complexes were extracted from the PPI networks. [35] The complexes in the PPI networks were identified with the help of the database CORUM. [36] To find out related complexes, we gave each clique forming protein as query in the CORUM database. Then, all the proteins associated with a specific complex were determined by the in house algorithm. [37]

Results

Determination of differential expression (DE) genes

For PBMCs, 2466 genes were reported to have differential expression using ANOVA test (*p*-value <0.05) that 1024 genes were up-regulated and 1442 were down-regulated. The genes and corresponding *p*-values are listed in the supplementary material Table 1. In case of pancreatic beta cells, we used the data of by De´ cio L. Eizirik *et al*. in 2012 [7] in which 3068 genes were significantly modified by exposure to the pro-inflammatory cytokines IL-1B plus IFN-ɤ. From these, 1416 and 1652 were respectively up and downregulated.

Exploration of bilateral cooperation between immune cells and pancreatic beta cells in T1D via gene expression profiles

It was suggested that beta cells play active roles in inflammation as they can express chemokines and cytokines to attract effector immune cells during inflammation. [7] Given the concept of a "dialog" between pancreatic beta cells and invading macrophages and T cells in the course of insulitis, [6, 7] and no study has attempted to delineate it with systems approach, we therefore hypothesized that there may be found the bilateral relations between chemokines and cytokines genes expression between two circumstances (PBMCs/ beta cells). We found differentially expressed chemokines and cytokines in beta cells and their receptors in PBMCs. Table 1 summarizes the results, and Figure 2 illustrates this cooperation in the context of the PPI networks.

There are provided brief descriptions for each pair of discovered chemokines, cytokines and their receptors. To start with, CXCR2 is a chemokine receptor for chemokines of CXCL1, CXCL2, CXCL3 and CXCL5. This chemokine-receptor with mentioned chemokines mediated neutrophil migration to sites of inflammation. [38] Along with this, the study of Diana and Lehuen showed that macrophages and beta cells produced the chemokines CXCL1 and CXCL2 which recruited CXCR2-expressing neutrophils from the blood to the pancreatic islets during autoimmune diabetes in NOD mice. [39] The second, CXCR3 as a chemokine receptor is activated by three interferon-inducible ligands CXCL9, CXCL10 and CXCL11; interactions between this chemokine-receptor and these three chemokines induced the recruitment of T cells into inflammatory sites. [38] Antonelli and *et al*. study indicated that CXCL10/CXCR3 system had crucial role in the autoimmune process and destruction of viral infected beta-cells in T1D. [40] The third, CCR1 is a chemokine receptor that with their ligands, CCL3 and CCL5 mediated signal transductions which are critical for the recruitment of effector immune cells to the site of inflammation. [38] It has been proved from several mouse

models that CCL3 and CCL5 expressed in pancreatic islets and implicated in T1D development. [41] The fourth, CCR4 is a chemokine receptor for CCL22 that they also played a role in the trafficking of activated T lymphocytes to inflammatory sites. [38] There is reported that a pathogenic role for CCL22 was hypothesized based on reduced insulitis and diabetes frequencies in NOD mice treated with a neutralizing CCL22 antibody. [42] Additionally, there were some interleukins such as IL-6, IL-1A and IL-1B in beta cells and their receptors (IL6R, IL1R2 and IL1RAP) in PBMCs that all of them and their receptors have a major role in immune response especially in inflammation. With this in mind, there is evidence that interleukin 1 (IL‐1), in concomitant with tumor necrosis factor and interferon ɤ, induced apoptosis of pancreatic beta‐cells; clinical trials with IL‐1 antagonists have been initiated in patients with T1D. [43] In case of interleukin-6, Ryba-Stanislawowska *et al*. proposed an important regulatory role of IL-6 in the progression of diabetes and its complications. [44] As a last point, we also found interferon gamma (IFN-ɤ) and interferon β1 (IFN-β1) in PBMCs and their receptors (IFNɤR2 and IFNαR2) in beta cells.

Topological analyses of QQPPI networks

Our studied QQPPI networks were undirected and unweighted protein-protein interaction networks based on DE genes of both PBMCs and pancreatic beta cells in T1D. After removal of all orphan nodes, the QQPPI networks included 949 proteins and 1776 interactions in PBMCs and 1358 proteins and 3505 interactions in beta cells that they were used for further analysis. QQPPI networks have been studied by several topological parameters which gave more definitions about interactions network. Some global properties of the networks are shown in Table 2. The power law of node degree distribution is one of most important criteria of biological networks .The degree values approximately followed power law distributions ($P(k) = k^{-\lambda}$), with λ= 2.13 and λ=1.95 for the PBMCs and beta cells networks, respectively, Figure 3, which indicated that the QQPPI networks were scale-free. The number

of hubs and bottlenecks was 29 and 48 in PBMCs and 40 and 68 in pancreatic beta cells networks. The hub-bottleneck nodes in PBMCs and beta cells networks are illustrated in Figure 4. The list of all nodes, hubs and bottlenecks in PBMCs and beta cells along with their topological parameters as obtained from Cytoscape software are prepared in supplementary materials Table 2 and 3.

Identification of functional modules

To better understanding of the biological processes or molecular functions under gene expression of T1D, in this study, the PPI networks have been decomposed into 11 and 12 functional modules in PBMCs and pancreatic beta cells respectively by ClusterONE algorithm via Cytoscape. In case of PBMCs, the biological processes of inflammatory response, chemotaxis, defense response, immune response and the related pathways such as chemokine signaling pathway, cytokine-cytokine receptor interaction and signaling in immune system demonstrated the immune cells intermediation in the diseases pathogenesis. Response to DNA damage stimulus, an initiation of viral infection, and non-homologous end-joining (NHEJ) pathway were the other significant biological processes and pathway. Finally, mRNA metabolic process, RNA splicing, ATP metabolic process, modification-dependent macromolecule catabolic process, proteolysis, translational initiation, proteasomal ubiquitin-dependent protein catabolic process were the noticeable remaining biological processes; the relevant significant pathways included spliceosome, oxidative phosphorylation, metabolism proteins and proteasome (Table 3).

In case of beta cells, the majority of biological processes and pathways were related to immune response and apoptosis (Table 4). The more significant biological processes were chemotaxis, regulation of I-kappaB kinase/NF-kappaB cascade, antigen processing and presentation of peptide or polysaccharide antigen via MHC class I and antigen processing and presentation of peptide antigen via MHC class I. In this sense, Natural killer cell mediated

cytotoxicity, NOD-like receptor signaling, Chemokine signaling, B cell receptor signaling, T cell receptor signaling, cytokine-cytokine receptor and Toll-like receptor signaling pathway were the remarkable immune pathways. Apoptosis and p53 signaling were the apoptotic enriched pathways. Besides, there were some major biological processes such as regulation of protein modification process, positive regulation of DNA metabolic process, proteolysis, ubiquitin-dependent protein catabolic process, spliceosomes snRNP biogenesis, regulation of RNA metabolic process. Jak-STAT signaling and insulin signaling pathways were two noticeable pathways related to genetic information processing class. Positive regulation of cell proliferation, cell migration and cell communication were the last significant biological processes. Regulation of actin cytoskeleton and focal adhesion were two significant pathways for cellular processes class. The last remarkable pathway was neurotrophin signaling pathway that it established to be involved in T1D pathogenesis. [45]

Identification of complexes

Several three and four cliques were identified in the QQPPI networks using CFinder software. The corresponding complexes were retrieved from the CORUM database and shown in Table 5 and 6. For PBMCs, the identified complexes involved in many biological processes like protein processing, proteasomal degradation, stress response, protein binding, protease activator (ID: 38, 39, 181, 191, 192, 193, 194)), protein biosynthesis (ID: 306), RNA processing and RNA binding (ID: 351, 1181, 1332), translation initiation (ID:742, 1097), phosphate metabolism, transcription activation and protein modification (ID: 2601), transcription repression and DNA binding (ID: 2918), ribosome biogenesis (3055), cell cycle and RNA synthesis (ID: 5593), apoptosis (ID: 5623).

In case of pancreatic beta cells, these complexes mediated various biological functions such as protein processing, proteasomal degradation, stress response, protein binding, protease activator (ID: 38, 39, 181, 191, 192, 193, 194),), ribosome biogenesis and protein

biosynthesis (ID:306, 3055), RNA processing and RNA binding (ID:351, 1189, 1335, 5615), regulation of transcription (ID: 2084, 2086, 1335) , assembly of protein complexes (ID: 2242), protein binding and cell adhesion (ID: 2376, 2383, 5342), cell cycle, protein modification and cellular signaling and adaptive cell mediated response (T-cells) (ID: 2470, 2529, 2551), apoptosis, (ID: 2684, 4158, 5623), I-kappaB kinase/NF-kappaB cascade and cytokine activity(ID: 5193,5196, 5228, 5232, 5233, 5269, 5464, 5492), intracellular signaling cascade(ID: 5220, 5615), G-protein coupled receptor protein signaling pathway and cell migration(5342).

Identification of key markers for T1D

To identify key markers in QQPPI networks, we prepared the list of proteins which were hub-bottleneck in each QQPPI network, and then we chose the hub-bottleneck nodes that most of them existed in both identified functional modules and complexes in the networks. It has been established that hubs and bottlenecks paly pivotal role in networks and considered as biologically significant proteins. [46, 47] The key markers and their functions are listed in Table 7 and their locations in functional modules are shown in Figure 5.

Discussion

Given the complex genetics of T1D, and the notion that ongoing molecular mechanisms of the disease contribute to the processes mediated both at the immune system and at the pancreatic beta cell level, [7] we thus combined transcriptome and interactome data in PBMCs and pancreatic beta cells to explicate the underlying biological pathways and to decipher missing heritability of this complex disease, which may be stealth within the gene network.

Here, for the first time, we were able to show the bilateral relations of PBMCs and pancreatic beta cells considering differentially expressed chemokines, cytokines and their receptors in both circumstances via systems approach. Our results verified that beta cells are not passive victims during pathogenesis. Pathway enrichment analysis implicated that

Immune response, apoptosis, spliceosome and proteasome, pathways of protein synthesis were the most significant enriched pathways in both tissues. However, in PBMCs, class of metabolism (Oxidative phosphorylation) and phosphate metabolism complex (ID: 2601) comprised one of the significant enriched pathways in functional modules and biological processes in complexes. It has been proposed that chronic hyperglycemia itself may affect directly or indirectly the PBMCs gene expression profile of untreated diabetes. [48] PBMCs thus reflect the systemic metabolic changes as well as abnormal immune regulation.

We have prepared compendium annotations of most relevant key markers, hub-bottleneck nodes involved both in functional modules and complexes, in terms of their up/down expression to just describe their possible role in T1D pathogenesis. For PBMCs, the first key marker was the Y-box binding protein 1 (YBX1/YB1) in module 1 and complex (ID: 3055). It was downregulated and incorporated in enriched spliceosome pathway. It has been reported that YBX1 gene plays role in cytokines mRNA stability which is important in autoimmune diseases. [49] Besides, there is evidence by which revealed an important role for YB-1 as a regulator of PTP1B (prototypic protein tyrosine phosphatase) expression. PTP1B considered as a critical regulator of insulin- and cytokine-mediated signal transduction. [50] The second and third markers were Proteasome subunit alpha 1/3 (PSMA1/PSMA3) genes that involved in module 6 and proteasome complex, and they were upregulated. They were member of enriched proteasome pathway. The ubiquitin proteasome system has salient biological role in the antigen processing and immune response as it could potentially be involved in pathogenesis of many immunity-related diseases. [51] The fourth marker, DNA non-homologous end-joining repair gene (XRCC6) was downregulated and found in module 9 in which Non-homologous end-joining (NHEJ) was an enriched pathway and in complex (ID: 2918). XRCC6 encodes an enzyme involved in V(D)J recombination, [52] the process of genetic rearrangement in generating B cell receptor and T cell receptor diversity. The work of

Khanna *et al*. showed that proteomic defects in XRCC6 may cause not only lower double-strand break repair capacity, but also related to severe combination immune deficiency due to severely impaired variable division joining recombination capacity. [53] The last, SR (serine/arginine) protein kinase (SRPK1) gene, in module 4 and complex (ID: 3055), was upregulated. SRPK1 is activated early during apoptosis. The possible biological roles for SRPKs (1 and 2) are an involvement in signaling pathways governing apoptosis, alternative mRNA splicing, RNA stability, and possibly the generation of autoantibodies directed against splicing factors. [54]

In case of pancreatic beta cells, there were 5 key markers, CBL, SRC, PIK3R1, PLCG1 and SHC1 which mapped on module 1. E3 ubiquitin-protein ligase CBL (CBL) gene which located in complex (ID: 2529) inherently involves in pathway (cbl/cap pathway); this pathway is parallel one to insulin action for uptake glucose via GLUT4 translocator. Downregulation of this gene may be correlated to insulin resistance. [55] Non receptor tyrosine kinase (SRC) gene was upregulated and incorporated in complex (ID: 2470). There is study showed that inhibition of Src (c-Src) activation by Exendin-4 reduced endogenous ROS production and increased ATP production in diabetic GK rat islets. [56] Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) gene was downregulated and involved in complexes (ID: 2470, 2529, and 2551). Mutations in PIK3R1 as a regularity subunit caused primary immunodeficiencies especially antibody deficiencies (hypogammaglobinemia or agammaglobulinemia). [57] T1D has been reported in X-linked agammaglobulinemia (XLA) patients. [58] Phospholipase C, gamma-1 (PLCG1) gene was downregulated and involved in natural killer cell mediated cytotoxicity, the T-cell receptor signaling pathway, the Fc-epsilon RI signaling pathway and in complexes (ID: 2529, 2551). SHC-transforming protein-1 (SHC1) gene was upregulated and located in ErbB signaling pathway, chemokine signaling pathway and insulin signaling pathway. Upregulation of p46Shc which induced decreased

insulin signaling sensitivity has been reported in ShcP mice. [59] All of five markers were incorporated in ERBb signaling pathway. Moreover, CBL, SHC1 and PIK3R1 genes were involved in both natural killer cell mediated cytotoxicity and neurotrophin signaling pathway, and CBL, SHC1 and PIK3R1 were incorporated into insulin signaling pathway. Finally, ubiquitin-conjugating enzyme E2N (UBE2N/UBC13) in module 5 was downregulated. The results of Chang et al study showed that Ubcl13 played crucial role in maintenance of the in vivo immunosuppressive function of Treg cells and in inhibition of the conversion of Treg cells into TH1- and TH17-like effector T cells in a manner dependent on its downstream target IκB kinase (IKK) in mice. [60]

In summary, this study shows that network biology can be considered as an effectual approach to get knowledge about the underlying etiology of complex diseases such as T1D and that data integration is pivotal to such analyses. The analysis of PBMCs and pancreatic beta cells transcriptomes propagated the concept of dialog between pancreatic islets and immune system, which mediated by cytokines and chemokines signaling pathways. Centrality , modularity and clique analyses of the constructed networks resulted in identifying significant biological pathways and genes. Finally, this network-based analysis facilitated experimental identification of new diagnostic biomarkers and the development of therapeutic targets.

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Disclosure

The authors declare that there are no conflicts of interest.

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regulatory T cells and prevents their conversion into effector-like T cells. Nature immunology. 2012;13(5):481-90.

Figures' legends:

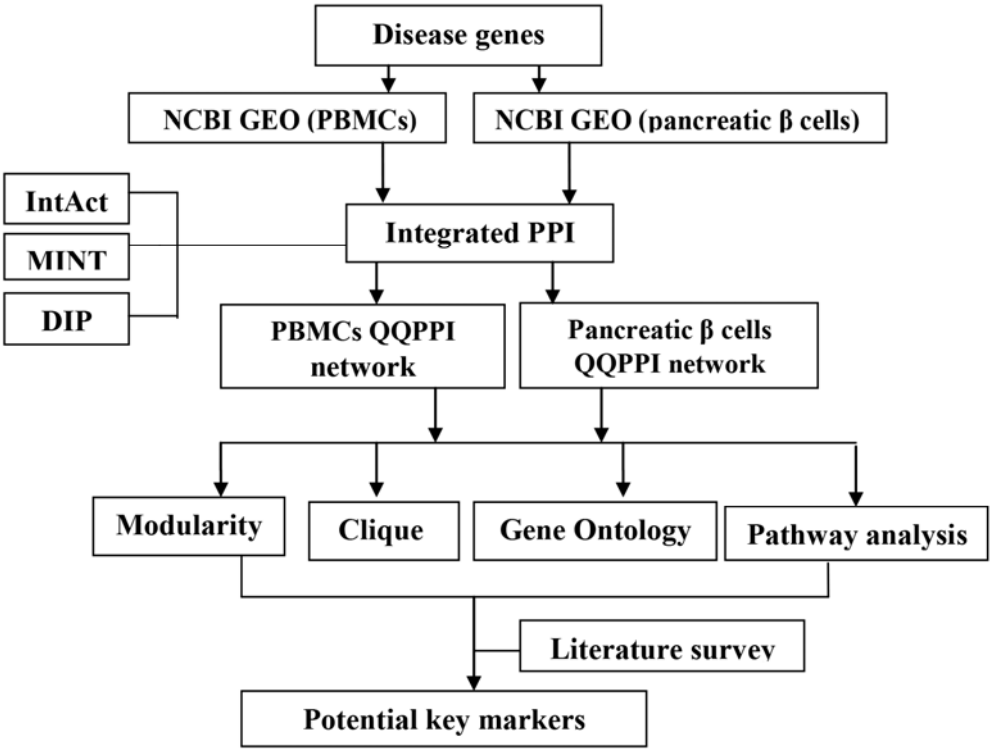


Figure 1. The study workflow.

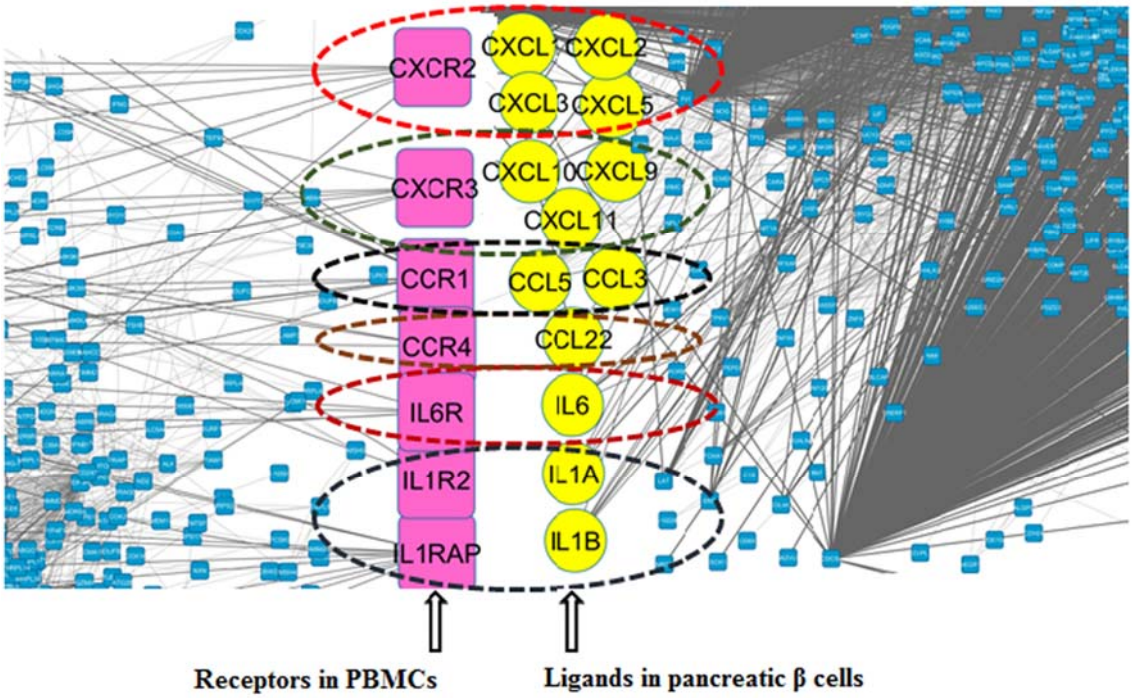


Figure 2. Bilateral cooperation between immune cells and pancreatic beta cells in early T1D.

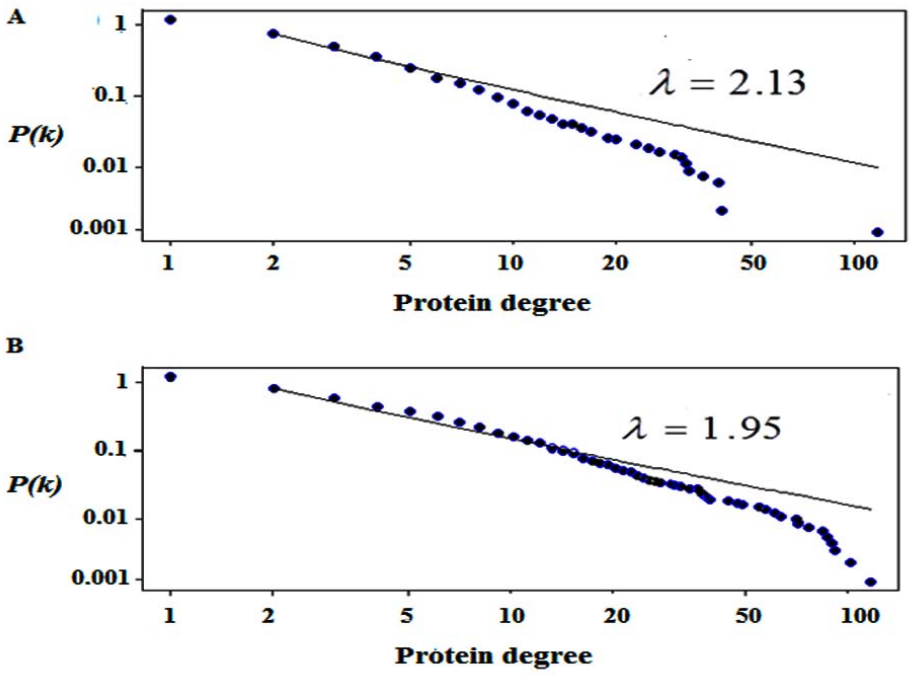


Figure 3. Power law distribution of node degree. (A) Degree distribution of QQPPI network in PBMCs. (B) Degree distribution of QQPPI network in pancreatic beta cells.

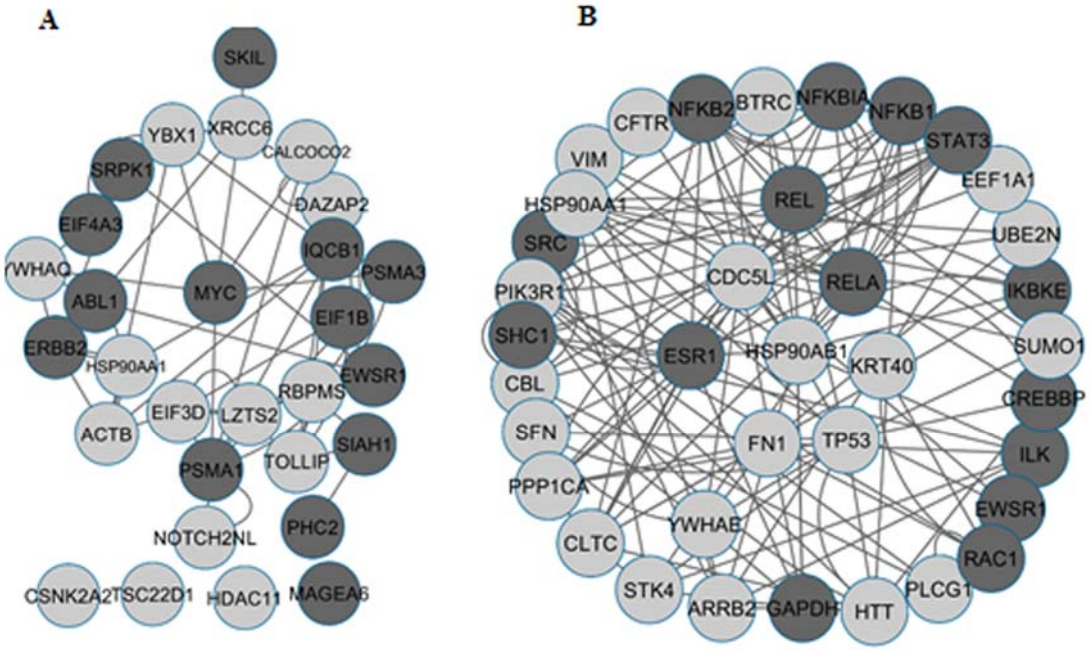
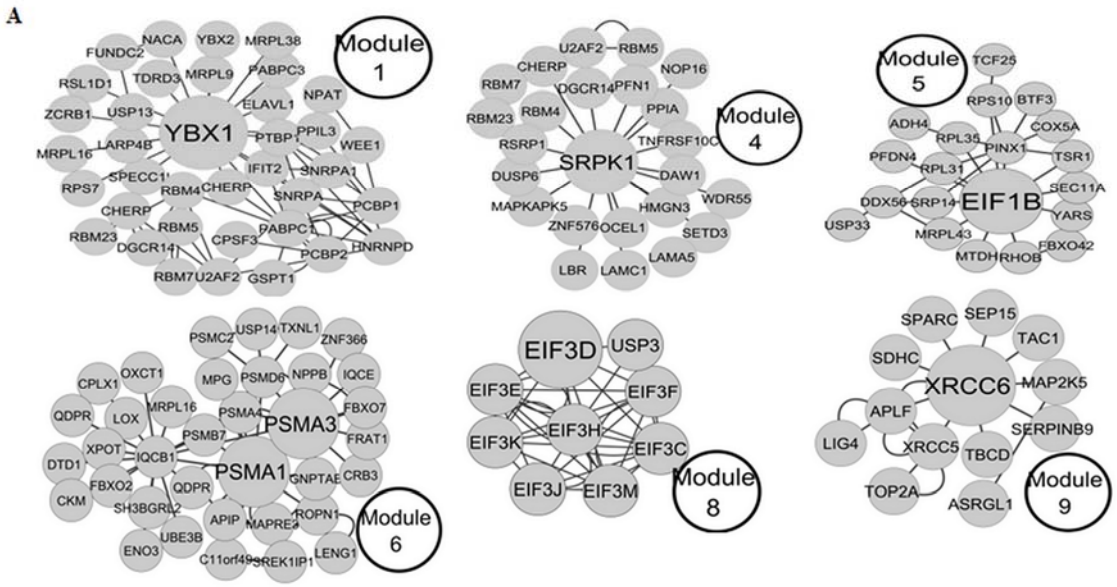


Figure 4. Hub-bottleneck nodes in (A) PBMCs, (B) pancreatic beta islets. Upregulated and downregulated genes are illustrated by dark gray and light gray colures.



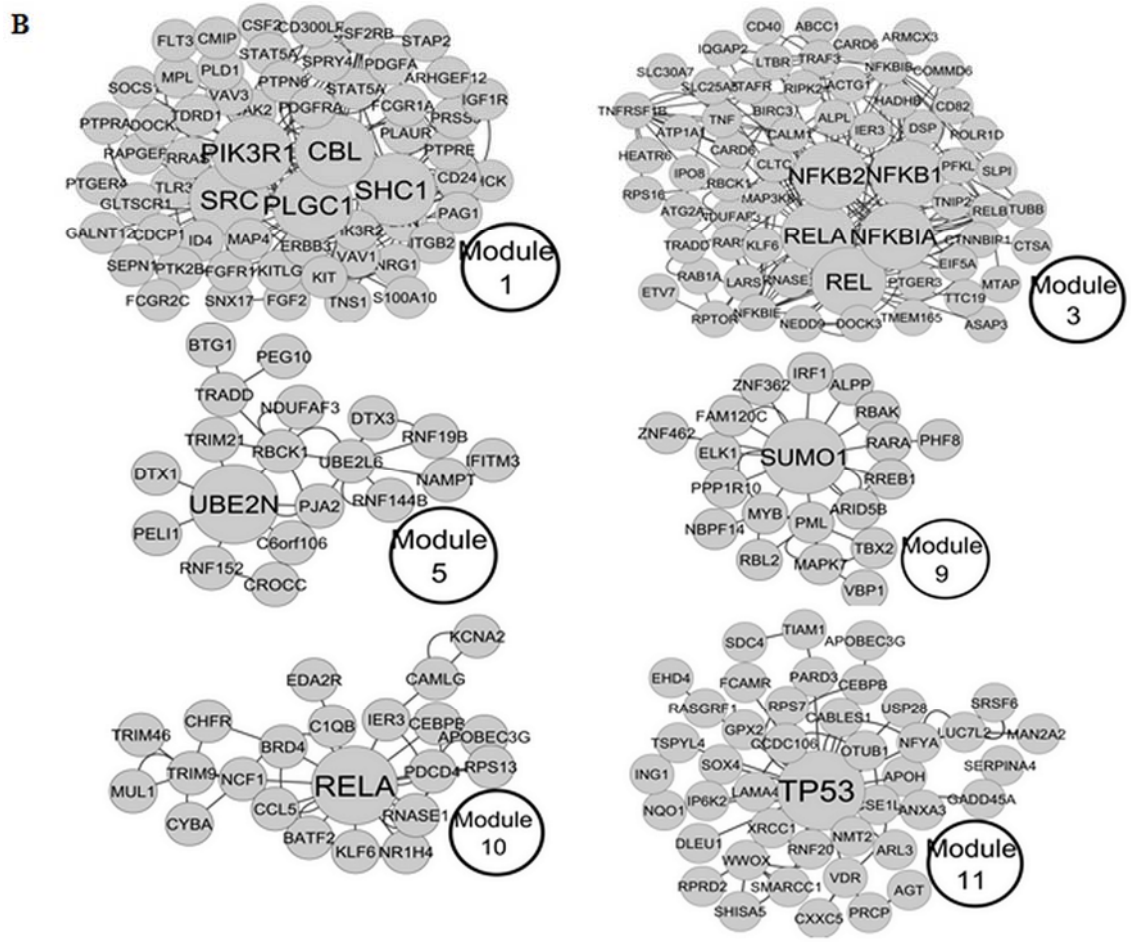


Figure 5. Functional modules involved key markers of (A) PBMCs**,** (B) pancreatic beta cells. Key markers were shown in bigger size than others.

Table 1 The list of differentially expressed ligand-receptor genes between pancreatic beta cells and PBMCs

PBMC (receptors)	Pancreatic beta cells (ligands)	Major functions
CXCR2	CXCl1,CXCL2,CXCl3,CXCL5	Neutrophil recruitment
CXCR3	CXCL9,CXCl10,CXCl11	Effector T cell recruitment
CCR1	CCL3,CCL5	Mixed leukocyte recruitment
CCR4	CCL22	T cell and basophil recruitment
IL6R	IL-6	Inflammation and B cell maturation
IL1R2,IL1RAP	IL1-A	Inflammatory processes and hematopoiesis
IL1R2,IL1RAP	IL1-B	Mediator inflammatory responses

Table 2 Global properties of the networks.

	Number of nodes	Number of edges	Average degree	Average betweenness	Average CC*	Average ASP*	Average CF*
PBMCs	949	1776	3.75	0.011	0.268	4.404	0.048
Beta cells	1358	3508	5.16	0.005	0.276	4.037	0.060

CC: closeness centrality, ASP: average shortest pathlength, CF: clustering coefficient

Table 3 The list of pathways enriched in modules for PBMCs.

Module ID	Pathway	*p*-value
M1	hsa03040: Spliceosome	1.6E-5
M2	hsa00190: Oxidative phosphorylation	7.4E-5
M5	REACT_1762: 3' -UTR-mediated translational regulation	2.7E-9
	REACT_17015: Metabolism of proteins	9.5E-8
	REACT_71: Gene Expression	1.1E-6
M6	hsa03050: Proteasome	1.1E-5
M7	hsa04062: Chemokine signaling pathway	1.8E-4
	hsa04060: Cytokine-cytokine receptor interaction	3.3E-4
	hsa05120: Epithelial cell signaling in Helicobacter pylori infection	5.9E-3
M9	hsa03450: Non-homologous end-joining	3.2E-4
M10	REACT_6900: Signaling in Immune system	7.1E-3

Table 4 The list of pathways enriched in modules for pancreatic beta cells.

Module ID	Pathway	*p*-value
M1	hsa04650: Natural killer cell mediated cytotoxicity	8.2E-6
	hsa04810: Regulation of actin cytoskeleton	8.2E-6
	hsa04630: Jak-STAT signaling pathway	6.7E-6
	hsa04012: ErbB signaling pathway	5.9E-6
	hsa04666: Fc gamma R-mediated phagocytosis	3.1E-6
	hsa04510: Focal adhesion	2.5E-5
	hsa04062: Chemokine signaling pathway	5.1E-4
	hsa04664: Fc epsilon RI signaling pathway	1.8E-4
	hsa04662: B cell receptor signaling pathway	1.6E-4
	hsa04660: T cell receptor signaling pathway	1.4E-4
	hsa04060: Cytokine-cytokine receptor interaction	4.3E-3
	hsa04910: Insulin signaling pathway	1.4E-2
	hsa04722: Neurotrophin signaling pathway	4.8E-2
M2	REACT_604:Hemostasis	3.8E-2
M3	hsa04920: Adipocytokine signaling pathway	7.0E-5
	hsa04621: NOD-like receptor signaling pathway	8.1E-5
	hsa04622: RIG-I-like receptor signaling pathway	9.6E-4
	hsa04620: Toll-like receptor signaling pathway	5.2E-3
	hsa04940: Type I diabetes mellitus	4.4E-8
	hsa04514: Cell adhesion molecules (CAMs)	2.3E-6
M6	hsa03050: Proteasome	1.4E-11
	hsa04612: Antigen processing and presentation	3.8E-3
M7	REACT_11052:Metabolism of non-coding RNA	1.2E-2
M8	hsa03440: Homologous recombination	1.4E-3
M11	hsa04115: p53 signaling pathway	3.4E-2
M12	hsa04115: p53 signaling pathway	6.3E-3
	REACT_578:Apoptosis	1.1E-4

Table 5 The identified complexes in PBMCs

Symbol	Complex
PSMA1, PSMA3, PSMA4, PSMB7, PSMB3, PSMC2, PSMD6	Proteasome (ID: 38, 39, 181, 191, 192, 193, 194)
RPS7, RPL35, RPL31,RPS10	Ribosome, cytoplasmic (ID:306)
EIF4A3, PABPC1, SNRPA, SNRPA1, AQR, RBM22, IGF2BP3	Spliceosome (ID:351)
EIF3C, EIF3D, EIF3F, EIF3H, EIF3E, EIF3K, EIF3J, EIF3M	eIF3 complex (ID:742, 1097)
EIF4A3, PABPC1, SNRPA, SNRPA1, AQR, RBM22, HNRNPA2B1, HNRNPM	C complex (ID:1181)
SRPK1, EWSR1, FUS, HNRNPM	Large Drosha complex (ID: 1332)
CCNT1, CDK9, MED1	P-TEFb-BRD4-TRAP220 complex1, (ID: 2601)
XRCC5, YY1, XRCC6	Ku antigen-YY1-alphaMyHC promoter complex (2918)
RPS7, YBX1, SRPK1, RPL35, RPL31, SLC25A5, SRP14, HNRNPM	Nop56p-associated pre-rRNA complex (ID: 3055)
LIN37, LIN54, RBBP4	LINC core complex (ID: 5589, 5593)
HSP90AA1, MME, MAP3K5	Ask1-HSP90-AKT1 complex (ID: 5623)

Table 6 The identified complexes in pancreatic beta cells

Symbol	Complex
PSMA3, PSMA6, PSMA7, PSMB1, PSMB7, PSMB8, PSMB9, PSMC4	Proteasome (ID: 38, 39, 181, 191, 192, 193, 194)
RPL11, RPL5, RPS13, RPS16, RPS4X	Ribosome, cytoplasmic (ID:306)
CDC5L, DDX17, DDX5, EIF4A3, PRPF8, SNRNP200, SNRPE, SNRPG, SRRM1	Spliceosome (ID:351)
CDC5L, DDX5, EIF4A3, HNRNPC, HNRNPM, PRPF8, SNRNP200, SNRPE, SNRPG, SRRM1	C complex spliceosome (ID:1189)
CDC5L, DYNC1H1, GCN1L1, PPP1CA, SRRM1	CDC5L complex (ID:1183)
CDC5L, DDB1, EEF1A1, PFKL, PRPF8, SNRNP200, TUBB	SNW1 complex (ID:1335)
NFKB1, NFKB2, RELA, RELB	NFKB1-NFKB2-REL-RELA-RELB complex (ID: 2084, 2086)
CALM1, HTT, TGM2	TGM2-HD-CALM1 complex (ID: 2242)
FN1, ITGB3, ITGA5, TGM2	ITGA2B-ITGB3-FN1-TGM2 complex , ITGA5-ITGB1-FN1-TGM2 complex (ID: 2376, 2383)
BCAR1, ESR1, PIK3R1, SRC	p130Cas-ER-alpha-cSrc-kinase- PI3-kinase p85-subunit complex (ID: 2470)
CBL, PIK3R1, PLCG1, VAV1	LAT-PLC-gamma-1-p85-GRB2-CBL-VAV-SLP-76 signaling complex (ID: 2529)
PDGFRA, PIK3R1, PLCG1	PDGFRA-PLC-gamma-1-PI3K-SHP-2 complex, PDGF stimulated (ID: 2551)
BCL2L1, TP53	p53-Bcl-xL complex, DNA-damage induced (ID: 2684)
EEF1A1, HNRNPM, RPL11, RPL5, RPS13, RPS16, SLC25A5	Nop56p-associated pre-rRNA complex (ID: 3055)
CALM1, HSP90AA1, HSP90AB1	HSP90-FKBP38-CAM-Ca(2+) complex (ID: 4158)

NFKB1, NFKB2, NFKBIA, NFKBIB, NFKBIE, RELA, RELB, TNIP2, BTRC, IKBKE, GTF2I, MAP3K8, RPS13, IQGAP2, HSP90AA1, HSP90AB1	TNF-alpha/NF-kappa B signaling complex (ID: 5193, 5196, 5233, 5269)
IQGAP2, RELA, TNIP2	CHUK-IQGAP2-AKAP8L-RELA-TNIP2 complex (ID: 5220)
MAP3K8, RELA , TNIP2	REL-MAP3K8-RELA-TNIP2-PAPOLA complex (ID: 5228)
NFKB1, NFKB2, NFKBIA, NFKBIE, RELA, TNIP1, TNIP2	CHUK-NFKB2-REL-IKBKG-SPAG9-NFKB1-NFKBIE-COPB2-TNIP1-NFKBIA-RELA-TNIP2 complex (ID: 5230)
LMO1, RAC1	ELMO1-DOCK1-RAC1 complex (ID: 5342)
NFKB1, NFKBIA, RELA	I(kappa)B(alpha)-NF(kappa)Bp50-NF(kappa)Bp65 complex, IKBA-NF(kappa)Bp65-NF(kappa)Bp50 complex (ID: 5464,5492)
HDGF, NMI, PDCD4, YWHAE	Emerin complex 52 (ID: 5615)
HSP90AA1, HSP90AB1, MAP3K5	Ask1-HSP90-AKT1 complex (ID: 5623)

Table 7 Brief description of hub-bottleneck nodes in functional modules & complexes

Symbol	Fold change direction (1 increase,-1 decrease)	Module	Complex	Function
PBMCs				
XRCC6	-1	9	ID: 2918	ATP-dependent helicase involved involve in DNA non-homologous end joining (NHEJ)
YBX1	-1	1	ID: 3055	Mediates pre-mRNA alternative splicing regulation
SRPK1	1	4	ID: 3055	Protein kinase involved in the regulation of splicing
PSMA1	1	6	ID: 38, 39, 191	Proteasome's subunit 1 that it cleaves peptides in ATP/ubiquitin-dependent process
PSMA3	1	6	ID: 191, 192, 193	Proteasome's subunit 1 that it cleaves peptides in ATP/ubiquitin-dependent process
EIF1B	1	5		Probably involved in translation
EIF3D	-1	8	ID: 742, 1097	Participant in the initiation of protein synthesis
Beta cells				
CBL	-1	1	ID: 2529	Adapter protein that functions as a negative regulator of many signaling Pathways
SRC	1	1	ID: 2470	Non-receptor protein tyrosine kinase involved in signal transductions
PIK3R1	-1	1	ID: 2470, 2529, 2551	Binds to activated (phosphorylated) protein-Tyr kinases and acts as an adapter
PLCG1	-1	1	ID: 2529, 2551	Mediates the production of the DAG and IP3 which has an important role In signaling cascades
SHC1	1	1		Signaling adapter that couples activated growth factor receptors to signaling pathway
RELA (NFKB3)	1	3,10	ID: 5220, 5230	NF-kappa-B is a pleiotropic transcription factor and endpoint of a series of signal transduction

				events
REL	1	3		Proto-oncogene which regulate genes involve in apoptosis, inflammation, the immune response
NFKB2	1	3	ID: 2086, 5230	it functions as a central activator of genes involved in inflammation and immune function
NFKB1	1	3	ID: 2084, 5464	NF-kappa-B is a pleiotropic transcription factor and endpoint of a series of signal transduction events
NFKBIA	1	3	ID: 5230, 5494	it inhibits NF-kappa-B/REL complexes which are involved in inflammatory responses
UBE2N	-1	5		member of the E2 ubiquitin-conjugating enzyme family
SUMO1	-1	9		Ubiquitin-like protein that covalently attach to proteins
TP53	-1	11	ID: 2684	Acts as a tumor suppressor in many tumor types