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System network analysis of genomics and transcriptomics data identified type 1 diabetes-associated pathway and genes

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Abstract

Genome-wide association studies (GWASs) have discovered >50 risk loci for type 1 diabetes (T1D). However, those variations only have modest effects on the genetic risk of T1D. In recent years, accumulated studies have suggested that gene–gene interactions might explain part of the missing heritability. The purpose of our research was to identify potential and novel risk genes for T1D by systematically considering the gene–gene interactions through network analyses. We carried out a novel system network analysis of summary GWAS statistics jointly with transcriptomic gene expression data to identify some of the missing heritability for T1D using weighted gene co-expression network analysis (WGCNA). Using WGCNA, seven modules for 1852 nominally significant ($P < 0.05$) GWAS genes were identified by analyzing microarray data for gene expression profile. One module (tagged as green module) showed significant association ($P < 0.05$) between the module eigengenes and the trait. This module also displayed a high correlation ($r = 0.45$, $P < 0.05$) between module membership (MM) and gene significant (GS), which indicated that the green module of co-expressed genes is of significant biological importance for T1D status. By further describing the module content and topology, the green module revealed a significant enrichment in the “regulation of immune response” (GO:0050776), which is a crucially important pathway in T1D development. Our findings demonstrated a module and several core genes that act as essential components in the etiology of T1D possibly via the

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

regulation of immune response, which may enhance our fundamental knowledge of the underlying molecular mechanisms for T1D.

Introduction

Type 1 diabetes (T1D) is an autoimmune-mediated disease that is characterized by the selective damage of pancreatic beta cells, which consequently leads to the insufficient secretion of insulin [1]. T1D generally occurs in childhood and adolescence. At present, lifelong insulin replacement is the only therapy for T1D patients. Epidemiology surveys have showed that >300 million people were affected by diabetes in the world, and about 10–15% of them were T1D [2, 3]. Lifelong insulin management and disease-related complications imposes a heavy burden on society.

The exact pathogenesis of T1D is still largely unclear [4]. It is widely thought that interaction of susceptibility genes and environmental factors triggers and promotes the disease onset. T1D has a strong cluster in the family, a monozygotic and dizygotic twin study estimated the heritability of T1D is approximately 72% [5]. The human leukocyte antigen (HLA) region, located on chromosome 6p21, was recognized as the major risk factor for T1D and explains about 50% of the genetic variations for the risk of T1D [6]. Over the past decade, >50 non-HLA variants associated with T1D were uncovered by genome-wide association studies (GWASs) [7, 8]. However, those variations identified by GWASs only have contributed to modest effects on the genetic risks for T1D [4]. The missing heritability is still to be discovered.

Accumulated evidence has suggested that part of those missing heritability may due to gene–gene interactions [9, 10]. Gene–gene interactions have explained a portion of the missing heritability but were commonly difficult to be detected under a low statistical power [9]. It was suggested that integrating genomics data with transcriptomics data to perform system network analysis through weighted gene co-expression network analysis (WGCNA) could improve disease gene detection power by grouping genes of individually small effect together for testing and search functional genes harboring nominally significant association ($P < 0.05$) in GWAS [11]. WGCNA is a powerful method to detect gene connections by exploring co-expression genes for complex diseases [12], which overcomes the main limitation of most genetics studies concentrating only on individual genes with significant statistics in GWAS. It is useful to perform WGCNA to assess gene co-expression similarity to enhance our knowledge of molecular processes and identify novel intervention targets for the complex diseases such as T1D.

WGCNA has been successfully applied to identify disease-associated biological modules and genes, including some recent work from our team [13, 14]. For example, both of He et al. and Chen et al. have successfully identified significant modules and genes associated with bone mineral density (BMD) using network-based analysis [13, 14], which laid a technical foundation for our work here. The first WGCNA study for T1D was performed by Riquelme et al. [15], which established two co-expression networks, respectively, from T1D and healthy controls to compare the expression difference using public microarray data.

While, our study first combined GWAS data with gene expression data to perform system network analyses, which may be more comprehensive to identify co-expression genes.

In recent years, more and more studies have focused on elucidating the importance of the immune component in T1D development [16, 17]. In our current study, we integrated the largest T1D genomics data with public gene expression data from newly diagnosed T1D patients vs normal controls derived from peripheral blood mononuclear cells (PBMCs) to perform system network analysis by using WGCNA. PBMCs are appropriate to assess immune processes of T1D pathogenesis in which the important immunological biomarkers can be detected [18]. Our findings may help us to better understand of T1D genetics and may also provide potential intervention molecular targets for the treatment of T1D.

Results

Discovering interesting modules associated with T1D

A total of 2,048,237 single-nucleotide polymorphisms (SNPs) from T1D GWAS data with associated P -value were annotated to 17,666 genes with gene-based P -value by using Versatile Gene-based Association Study-2 (VEGAS-2) [19]. Genes with $P < 0.05$ were selected from GWAS data, containing 1852 of the 17,666 genes. The 1852 nominally significant GWAS genes set with corresponding probes were picked up from the gene expression profile. There were 3694 significant probes left for WGCNA analysis. By using WGCNA, seven distinct gene modules were identified based on hierarchical clustering dendrogram, presented in Fig. 1. The module size ranges from 40 (red module) to 1269 (blue module) genes. Next, we tested the association between the individual module and T1D status through the correlation of module eigengenes and T1D. Interestingly, the green module showed significant evidence ($P < 0.05$) associated with T1D, as seen in Fig. 2. Finally, the correlation of gene significant (GS) and module membership (MM) was generated to evaluate potential biological function of the interesting green module. As was shown in Fig. 3, the significant correlation ($r = 0.45$, $P = 0.0019$) between GS and MM was presented in the green module, which means that genes in the green module were not only significantly associated with T1D status but also are highly co-expressed.

Further exploration of the green module

To summarize the network results, we evaluated those core genes of the interesting green module from three aspects: top hub genes ordered by intramodular connectivity (K_{in} is described by the number of genes relating to a given gene), top network genes ordered by GS and top GWAS genes ordered by GWAS P -value, as shown in Table 1. The top hub genes include *CAPZB*, *YWHAZ*, and *TKT*, the top network genes consist of *TPPI*, *TKT*, and *RBM17*, the top GWAS genes involve *PTPN11*, *HCG11*, and *MLLT1*. Among those genes, the expression of *YWHAZ* was reduced in T1D conditions and this gene influences inflammation by inhibiting T-cell trafficking to inflamed tissue [20]. The *TPPI* gene is one of the top network genes and ranked the first on GS. *TPPI* gene was associated with rheumatoid arthritis (RA) [21], which suggested that *TPPI* may be a potential candidate gene for T1D, as T1D and RA were reported to have some common susceptibility loci [22]. In addition, *TPPI* may indirectly influence metabolism possibly by regulating telomere

length [23]. Moreover, various types of research have indicated that *TKT* might prevent the progress of complications of diabetes in animal experiments by inhibiting nuclear factor (NF)-kappa B signaling [24, 25]. The *PTPN11* gene was a known susceptibility gene for T1D reported by a previous GWAS [26], which was validated by our study. However, the other top genes *HCG11* and *MLLT1* (ranked on the level of statistical significance in the GWAS), *CAPZB* (based on K.in), as well as *RBM17* (ranked on GS) have so far no evidence of being associated with T1D in previous studies, which were novel candidates discovered by our present work.

The green module network visualization was conducted by VisANT, containing all the genes of the green module (Supplement Fig. 1). In a network, a node represents one gene, edges represent the related connections to a gene. Red nodes represent genes that are enriched in “regulation of immune response”, black nodes represent the top GWAS genes, yellow nodes mean the top hub genes, and the top GS genes are signed to blue. Forty-five nodes and 990 edges (representing potential interactions among the gene nodes) were included in the green module network.

Gene Ontology (GO) enrichment analysis of the green module was performed following the visualization step. Significant GO term (GO: 0050776) was observed in the “regulation of immune response” pathway (Table 2). Interestingly, this result showed that the regulation of immune response pathway might act as an essential component in the etiology of T1D, which was consistent with the result from previous studies [16, 27]. The regulation of immune response pathway includes 11 genes (*PTPN11*, *SKAP2*, *TAB2*, *ITGB1*, *ITGB2*, *RAC2*, *CD36*, *CDC37*, *PSMD13*, *PSMF1*, and *CMTM3*), the details of these genes are shown in Supplement Table 1. Among these genes, *PTPN11* and *SKAP2* related to T1D were reported by previous GWAS [26, 28], both of them were associated with the autoimmunity. Familial association studies provided a portion of the evidence that *TAB2* may indirectly regulate the NF-kappa B signaling and induce the apoptosis of pancreatic beta cells [29]. As the apoptosis of pancreatic beta cells is one of the most important characteristics of T1D [30], *TAB2* was known as a susceptibility gene for T1D. The following two genes, *ITGB1* and *ITGB2* also have been reported to have association with T1D in gene-based pathway analysis and NOD/ItJ mice experiment, respectively [31, 32]. Notably, *RAC2* was known to be highly associated with autoimmune disease [33], and a recent study of fine-mapping revealed its association with T1D [34]. However, for the rest five genes (*CD36*, *CDC37*, *PSMD13*, *PSMF1*, and *CMTM3*), we have not found studies reporting their roles in T1D.

The functional protein association networks of the green module were conducted by the STRING database. Overviewing the networks, two protein–protein interacting sub-networks were detected (Fig. 4). One of the co-expression subnetwork includes *PTPN11*, *RAC2*, *ITGB2*, *ITGB1*, *CD36*, *ZDHHC8*, *COMT*, *YWHAZ*, *SDHA*, *SDHB*, and *SDHC*. Interestingly, five (*PTPN11*, *RAC2*, *ITGB2*, *ITGB1*, and *CD36*) of them were involved in the immune response pathway and five known genes (*PTPN11*, *RAC2*, *YWHAZ*, *ITGB2*, and *ITGB1*) relevant to T1D were identified. Another co-expression subnetwork contained *FLI1*, *TPPI1*, and *SKAP2*.

Discussion

In our present study, we first performed a system network analysis study by combining evidence of summary GWAS statistics with disease-relevant transcriptome data for T1D. We successfully identified one module (tagged as green module) containing 45 interconnected genes and several core genes (e.g., *TPPI*, *RAC2*, and *PTPN11*) that are significantly associated with T1D. GO enrichment analysis indicated the interesting module enriched in the regulation of immune response pathway, revealing a close relationship between immune response and T1D. This finding is consistent with the result from another study in regulating the immune response [27]. The functional protein–protein association networks also demonstrated that five of the genes (which belong to immune response pathway) showed interaction evidence with each other. Furthermore, we have also identified several interesting genes, such as *TPPI*, which may be a promising novel candidate gene for T1D (which will be discussed in the following). Thus, our findings not only validated that immune response was very important for the etiology of T1D but also identified novel potential candidate genes for T1D. Our work may offer some valuable evidence for a better understanding of T1D genetics and thus provide potential molecular intervention targets for the treatment of T1D.

System network analysis is a useful biological approach to identify co-expression genes for complex diseases. The highlighted advantage of this approach is that it can find potential novel candidate modules and genes with functional features based on co-expression similarities, which differs from other conventional pathway analysis that focuses on searching gene lists for the known molecular mechanism in a database. System network analysis with transcriptome gene expression data derived from disease-relevant cells or tissues provide potential functional information and may improve statistical power by clustering those co-expression genes (each may with small effects) for group testing, which may make up for some of the limitations of GWAS. Take this into account, we performed system network analysis study via WGCNA for combining summary GWAS statistics with gene expression profile. In our study, we identified an interesting module that showed a positive association with T1D. GO and pathway analyses further verified this specific process. Eleven of the 45 genes (*PTPN11*, *SKAP2*, *TAB2*, *ITGB1*, *ITGB2*, *RAC2*, *CD36*, *CDC37*, *PSMD13*, *PSMF1*, and *CMTM3*) were significantly enriched in the regulation of immune response, including several well-known genes that were related to T1D in previous studies (*PTPN11*, *SKAP2*, *RAC2*, *TAB2*, and *ITGB1*) [26, 28, 29, 31, 34]. A previous study has also showed that both of innate and adaptive immune response were involved in the development of T1D [17]. Dysfunction of immune tolerance expands the activation of disease-mediated autoreactive T cells infiltrating pancreas islet, which simultaneously leads to the release of cytokines that directly promote the death of beta cells [27, 35]. Our results suggested the green module and its genes especially those genes that regulate immune response may play essential roles in the etiology of T1D.

It is worth noting that *TPPI* was the top network gene in the green module and ranked first by GS (the correlation of individual gene and T1D from transcriptomics data). This gene is located on chromosome 11p15.4. The protein encoded by this gene is a member of shelterin complex, which plays an important role in the regulation of telomere length. An early study

has revealed that the telomere length from patients with T1D was significantly shorter than that of control subjects [36]. Additionally, previous studies have reported that telomere length was a biomarker of negative effects of oxidative stress and inflammation [37], especially a recent study showed that shelterin complex proteins may be involved in indirect regulation of metabolism [23]. Moreover, *TPPI* showed interaction evidence with *SKAP2* in the functional protein association network, and *SKAP2* has already been reported to be associated with T1D and RA [22]. Studies have also shown that part of the missing heritability may be due to gene–gene interaction or genes with individual small effect [9]. As described above, *TPPI* and *SKAP2* showed co-expression relationship with each other, thus *TPPI* may be also associated with T1D. A study of RA has found that decreasing expression of *TPPI* was observed in RA patients and it had a negative correlation with autoantibodies [21]. T1D and RA are two auto-immunity disease and were reported to share some susceptibility loci, such as *SKAP2* [22]. Although *TPPI* has not been directly reported to be a T1D gene in previous genetics studies, our study suggested its role in the etiology of T1D possibly by indirectly regulating telomere length and association with RA, which may enhance our understanding of the underlying molecular mechanisms in T1D. Based on our results, as well as combining the genetic background of T1D and RA with the association between *TPPI* and RA in previous studies, which all suggested that *TPPI* may be a promising new candidate gene for T1D. However, the specific mechanisms of *TPPI* for T1D are unknown and need to be further investigated.

RAC2 was the eighth hub gene and enriched in the immune response pathway according to the result of GO enrichment analysis. This gene, located on chromosome 22q13.1, belongs to Rho family and encodes small GTP binding proteins. *RAC2* was a well-known specific hematopoietic protein in the regulation of transcriptional activation and cytoskeletal reorganization among cells development, particularly in immune cells [38]. A recent study for fine mapping of T1D loci was performed using ImmunoChip, which revealed *RAC2* as a T1D gene [34]. Early research indicated that deficiency of *RAC2* resulted in the decrease of T-cell proliferation in cell experiment [39]. *RAC2* also plays a vital role in inflammation, namely secreting reactive oxygen species and producing cyclooxygenase-2 [33]. In addition, both the elicitation of immune response and immune tolerance is regulated by *RAC2* [40]. As we know, inflammatory process and immune response are key factors for developing T1D [16, 35]. Thus, our study demonstrated that *RAC2* may act as a significant player in the mechanism of T1D onset.

The *PTPN11/SHP2* gene, located on chromosome 12q24.13, encodes the protein tyrosine phosphatase (PTP). *PTPN11/SHP2* was a specific candidate gene for T1D, which involved in the regulation of insulin and immune signaling [41]. Recent studies have demonstrated that *PTPN11/SHP2* influenced immune response by mediating the effects of cytokine and inhibitory receptor signaling processes, such as downregulating T-cell adhesion processes and then inhibiting T-cell activation [42]. In addition, another study reported that increasing *PTPN11/SHP2* expression positively regulated mitogen activated protein kinase (MAPK) signaling and consequently upregulated the expression of arginase II, therefore reducing the cellular nitric oxide (NO) to accelerate insulin-mediated leukocyte adhesion and leading to consequent vascular complications of diabetes [43]. Hence, our study also identified

PTPN11 as a susceptibility functional gene for T1D, which verified the result of the previous GWAS [26].

Despite the above interesting findings, our study may have a few limitations. For example, the sample size of the microarray expression data that we used was not very large. Although the gene expression sample size is relatively small, the transcriptome data that obtained from PBMC can directly exhibit the expression difference of immune state in T1D patients and normal controls [44]. The PBMC were also widely used in functional studies for T1D. In addition, our study did not validate all the genes that were discovered in the original GWAS, because the network analysis is a functional biological approach for identifying the missing heritability of T1D by considering the gene–gene interactions in specific (and not every) disease-relevant cells or tissues. Further replication studies for a larger sample size of the transcriptome data and biological experiments are needed to verify our results.

In conclusion, through GWAS statistics data joined with disease-relevant transcriptome data to perform system network analysis using WGCNA, we successfully identified one module (green module) and several genes (e.g., *TPPI*, *RAC2*, and *PTPN11*) that may be the potential functional association with T1D. The associated genes of the interesting green module were highly enriched in the regulation of immune response pathway that provided biologically valuable clues in support of the association between those significant genes and T1D.

Materials and methods

Genomics data preparation and processing

The genomics data we used for analysis were publicly available summary GWAS statistics for T1D, which was the largest meta-analysis from Bradfield et al. [7]. The data consist of five constituent studies: T1DGC, GoKinD, DCCT-EDIC, WTCCC, and CHOP-McGill and include 26,890 individuals (9934 cases/16956 controls). The samples were described in detail in the previously study [7]. The summary GWAS statistics were downloaded from <http://www.t1dbase.org/>. The GWAS SNPs with associated *P*-values were converted to relevant genes and corresponding *P*-values for genes using VEGAS-2 [19], which is a comprehensive web platform that can perform the gene-based and pathway-based analyses on summary GWAS statistics data. Genes with *P* < 0.05 were considered to be nominally significant for further analyses.

Transcriptomics data preparation and processing

The transcriptomics data set derived from public microarray data were downloaded from NCBI Gene Expression Omnibus (GEO) database (access number: GSE55100). In the previous study [45], PBMC were collected from 12 newly diagnosed T1D patients (17.50 ± 3.68 years) and 10 normal age-matched controls (18.70 ± 1.16 years). mRNA was extracted and complementary DNA was performed by using Affymetrix Human Genome U133 Plus 2.0 Array [HG-U133_Plus_2] platform. T1D patients were diagnosed according to 2011 American Diabetes Association (ADA) criteria [46]. Subjects did not meet the inclusion criteria were excluded, as the previous study described [45]. The downloaded

Affymetrix CEL files were normalized by R language Affy package. We selected the significant probes for analysis from expression profile based on the nominally significant genes of GWAS ($P \leq 0.05$).

Weighted gene co-expression network analysis

We chose the nominally significant genes of GWAS ($P \leq 0.05$) to build the co-expression network by using R language WGCNA package. The detail WGCNA tutorials can be found in <https://labs.genetics.ucla.edu/horvath/CoexpressionNetwork/Rpackages/WGCNA/Tutorials/index.html>. The WGCNA algorithm was based on the previous studies [12, 14], and the main steps were briefly summarized as follows: first, a co-expression matrix was established through calculating correlations among significant genes and converted the co-expression matrix into adjacency matrix using soft threshold power. We chose power = 12 for our network construction as it was appropriate for meeting the criterion of the resulting adjacency matrix approximating scale-free topology (Supplement Fig. 2). Then, a topological overlap matrix (TOM) was generated by the adjacency matrix. TOM is defined as the connection between genes, and also represents the strength of gene–gene interactions. Next, the hierarchical clustering dendrogram was constructed according to a dissimilarity TOM (1–TOM), modules were grouped with tightly connected genes. Each module was arranged to a different color.

Module eigengenes were obtained from the first principal component of co-expression matrix for modules. For finding significant gene modules, we created module–trait relationships to detect the correlation between module eigengenes and T1D trait. GS was determined by the correlation of each gene and T1D from transcriptomics data. MM was regarded as the correlation of module eigengenes and individual gene's expression profiles, MM is a parameter to evaluate the cohesion of genes to a module. The higher correlation of GS and MM, the more important the genes have for the trait and module. Finally, the submodule network visualization was performed by VisANT [47], which includes all nodes (for genes) and edges of the interesting module.

GO enrichment analysis for the interesting module

To assess whether the interesting module was biological and functional relevant to T1D, GO enrichment analysis (<http://geneontology.org/page/go-enrichment-analysis>) was performed to identify relevant pathways within which those genes in the interesting module were enriched. For further exploring the functional interactions between proteins encoded by genes in the interesting module, functional protein association networks were established by STRING database (<https://string-db.org/>) [48].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Atkinson M, Eisenbarth G, Michels A. Type 1 diabetes. *Lancet* 2014;383:69–82. [PubMed: 23890997]
2. Sherwin R, Jastreboff AM. Year in diabetes 2012: the diabetes tsunami. *J Clin Endocrinol Metab* 2012;97:4293–301. [PubMed: 23185035]
3. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. *Lancet* 2016;387:2331–9. [PubMed: 27302272]
4. Groop L, Pociot F. Genetics of diabetes – are we missing the genes or the disease? *Mol Cell Endocrinol* 2014;382:726–39. [PubMed: 23587769]
5. Kyvik KO, Green A, Beck-Nielsen H. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *BMJ* 1995;311:913–7. [PubMed: 7580548]
6. Clayton DG. Prediction and interaction in complex disease genetics: experience in type 1 diabetes. *PLoS Genet* 2009;5: e1000540. [PubMed: 19584936]
7. Bradfield JP, Qu HQ, Wang K, Zhang H, Sleiman PM, Kim CE, et al. A genome-wide meta-analysis of six type 1 diabetes cohorts identifies multiple associated loci. *PLoS Genet* 2011;7:e1002293. [PubMed: 21980299]
8. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 2009;41:703–7. [PubMed: 19430480]
9. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature* 2009;461:747–53. [PubMed: 19812666]
10. Li M, Wei C, Wen Y, Wang T, Lu Q. Detecting gene-gene interactions associated with multiple complex traits with U-statistics. *Curr Genom* 2016;17:403–15.
11. Farber CR. Systems-level analysis of genome-wide association data. *G3 (Bethesda)* 2013;3:119–29. [PubMed: 23316444]
12. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform* 2008;9:559.
13. He H, Zhang L, Li J, Wang YP, Zhang JG, Shen J, et al. Integrative analysis of GWASs, human protein interaction, and gene expression identified gene modules associated with BMDs. *J Clin Endocrinol Metab* 2014;99:E2392–9. [PubMed: 25119315]
14. Chen Y, Guo Y, He H, Lin X, Wang X, Zhou R, et al. Integrative analysis of genomics and transcriptomedata to identify potential functional genes of BMDs in females. *J Bone Mineral Res* 2016;31:1041–9.
15. Riquelme Medina I, Lubovac-Pilav Z. Gene co-expression network analysis for identifying modules and functionally enriched pathways in type 1 diabetes. *PLoS One* 2016;11:e0156006. [PubMed: 27257970]
16. Noble JA. Immunogenetics of type 1 diabetes: a comprehensive review. *J Autoimmun* 2015;64:101–12. [PubMed: 26272854]
17. Lehuen A, Diana J, Zaccane P, Cooke A. Immune cell crosstalk in type 1 diabetes. *Nat Rev Immunol* 2010;10:501–13. [PubMed: 20577267]
18. Axelsson S, Faresjo M, Hedman M, Ludvigsson J, Casas R. Cryopreserved peripheral blood mononuclear cells are suitable for the assessment of immunological markers in type 1 diabetic children. *Cryobiology* 2008;57:201–8. [PubMed: 18761006]
19. Mishra A, Macgregor S. VEGAS2: software for more flexible gene-based testing. *Twin Res Human Genet* 2015;18:86–91. [PubMed: 25518859]
20. Chimen M, McGettrick HM, Aptá B, Kuravi SJ, Yates CM, Kennedy A, et al. Homeostatic regulation of T cell trafficking by a B cell-derived peptide is impaired in autoimmune and chronic inflammatory disease. *Nat Med* 2015;21:467–75. [PubMed: 25894827]

21. Qing Y, Zhou J, Zhao M, Xie W, Yang Q, Xing Y, et al. Altered expression of TPP1 in fibroblast-like synovial cells might be involved in the pathogenesis of rheumatoid arthritis. *Rheumatol Int* 2012;32:2503–10. [PubMed: 21833529]
22. Kiani AK, Jahngir S, John P, Bhatti A, Zia A, Wang X, et al. Genetic link of type 1 diabetes susceptibility loci with rheumatoid arthritis in Pakistani patients. *Immunogenetics* 2015;67:277–82. [PubMed: 25904084]
23. Martinez P, Gomez-Lopez G, Garcia F, Mercken E, Mitchell S, Flores JM, et al. RAP1 protects from obesity through its extratelomeric role regulating gene expression. *Cell Rep* 2013;3:2059–74. [PubMed: 23791526]
24. Babaei-Jadidi R, Karachalias N, Ahmed N, Battah S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. *Diabetes* 2003;52:2110–20. [PubMed: 12882930]
25. Hammes H, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, et al. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 2003;9:294–9. [PubMed: 12592403]
26. Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, et al. Genome-wide 414 association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78. [PubMed: 17554300]
27. Hojny GF. Mechanisms that regulate peripheral immune responses to control organ-specific autoimmunity. *Clin Dev Immunol* 2011;2011:1–9.
28. Reddy MPL, Wang H, Liu S, Bode B, Reed JC, Steed RD, et al. Association between type 1 diabetes and GWAS SNPs in the southeast US Caucasian population. *Genes Immun* 2011;12:208–12. [PubMed: 21270831]
29. Owerbach D, Pina L, Gabbay KH. A 212-kb region on chromosome 6q25 containing the TAB2 gene is associated with susceptibility to type 1 diabetes. *Diabetes* 2004;53:1890–3. [PubMed: 15220215]
30. Tomita T. Apoptosis of pancreatic beta-cells in type 1 diabetes. *Bosn J Basic Med Sci* 2017;17:183–93. [PubMed: 28368239]
31. Evangelou M, Smyth DJ, Fortune MD, Burren OS, Walker NM, Guo H, et al. A method for gene-based pathway analysis using genome wide association study summary statistics reveals nine new type 1 diabetes associations. *Genet Epidemiol* 2014;38:661–70. [PubMed: 25371288]
32. Glawe JD, Patrick DR, Huang M, Sharp CD, Barlow SC, Kevil CG. Genetic deficiency of Itgb2 or ItgaL prevents autoimmune diabetes through distinctly different mechanisms in NOD/LtJ mice. *Diabetes* 2009;58:1292–301. [PubMed: 19223596]
33. Sironi M, Guerini FR, Agliardi C, Biasin M, Cagliani R, Fumagalli M, et al. An evolutionary analysis of RAC2 identifies haplotypes associated with human autoimmune diseases. *Mol Biol Evol* 2011;28:3319–29. [PubMed: 21680873]
34. Onengut-Gumuscu S, Chen W, Burren O, Cooper NJ, Quinlan AR, Mychaleckyj JC, et al. Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. *Nat Genet* 2015;47:381–6. [PubMed: 25751624]
35. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulinitis and β -cell loss in type 1 diabetes. *Nat Rev Endocrinol* 2009;5:219–26. [PubMed: 19352320]
36. Jeanclos E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH, et al. Shortened telomere length in white blood cells of patients with IDDM. *Diabetes* 1998;47:482–6. [PubMed: 9519758]
37. Shalev I. Early life stress and telomere length: investigating the connection and possible mechanisms: a critical survey of the evidence base, research methodology and basic biology. *Bioessays* 2012;34:943–52. [PubMed: 22991129]
38. Pai SY, Kim C, Williams DA. Rac GTPases in human diseases. *Dis Markers* 2010;29:177–87. [PubMed: 21178276]
39. Yu H, Leitenberg D, Li B, Flavell RA. Deficiency of small GTPase Rac2 affects T cell activation. *J Exp Med* 2001;194:915–26. [PubMed: 11581314]
40. Ramaswamy M, Dumont C, Cruz AC, Muppidi JR, Gomez TS, Billadeau DD, et al. Cutting edge: Rac GTPases sensitize activated T cells to die via Fas. *J Immunol* 2007;179:6384–8. [PubMed: 17982024]

41. Mustelin T, Vang T, Bottini N. Protein tyrosine phosphatases and the immune response. *Nat Rev Immunol* 2005;5:43–57. [PubMed: 15630428]
42. Salmond RJ, Alexander DR. SHP2 forecast for the immune system: fog gradually clearing. *Trends Immunol* 2006;27:154–60. [PubMed: 16458607]
43. Giri H, Muthuramu I, Dhar M, Rathnakumar K, Ram U, Dixit M. Protein tyrosine phosphatase SHP2 mediates chronic insulin-induced endothelial inflammation. *Arterioscler Thromb Vasc Biol* 2012;32:1943–50. [PubMed: 22628433]
44. Kaizer EC, Glaser CL, Chaussabel D, Banchereau J, Pascual V, White PC. Gene expression in peripheral blood mononuclear cells from children with diabetes. *J Clin Endocrinol Metab* 2007;92:3705–11. [PubMed: 17595242]
45. Yang M, Ye L, Wang B, Gao J, Liu R, Hong J, et al. Decreased miR-146 expression in peripheral blood mononuclear cells is correlated with ongoing islet autoimmunity in type 1 diabetes patients. *J Diabetes* 2015;7:158–65. [PubMed: 24796653]
46. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2011;34(Suppl 1):S62–9. [PubMed: 21193628]
47. Hu Z, Snitkin ES, DeLisi C. VisANT: an integrative framework for networks in systems biology. *Brief Bioinform* 2008;9:317–25. [PubMed: 18463131]
48. Hillenmeyer S, Davis LK, Gamazon ER, Cook EH, Cox NJ, Altman RB. STAM: STRING-assisted module search for genome wide association studies and application to autism. *Bioinformatics* 2016;32:3815–22. [PubMed: 27542772]

Fig. 1. WGCNA co-expression network was generated for T1D GWAS genes. The hierarchical clustering dendrogram was established for 1852 nominally associated GWAS genes. Genes were clustered in modules based on a dissimilarity measure ($1 - \text{TOM}$). Each line represents an individual gene. The branches correspond to modules of tightly connected genes

Fig. 2. Module-trait relationships of the network. Each row represents a module eigengenes and column represents trait. Each cell contained the corresponding correlation and *P*-value. The table was colored by correlation according to the color legend. The green module showed significant correlation with T1D

Fig. 3.

The scatter plot of MM and GS. Shown is the scatter plot of module membership (MM) and gene significant (GS) in the interesting green module. The high correlation between MM and GS was observed in this module

Fig. 4. The functional protein association networks of the green module. The functional protein association networks of the green module, nodes represent protein, edges represent protein–protein association, line color represents types of interaction evidence (e.g., experimentally determined, gene fusions and so on). All of the interacting proteins with a interaction score 0.4 (based on the previous study, the interaction score 0.4 was regarded as medium confidence)

Table 1

Network top genes of the green module

Gene	Description	Unadjusted GWAS	P-value	r	r P-value	K.in rank
Top GWAS genes (ordered by GWAS)						
<i>PTPN11</i>	Protein tyrosine phosphatase, non-receptor type 11	3.02E-17		0.3	2.54E-01	18
<i>HCG11</i>	HLA complex group 11	3.11E-07		0.44	8.75E-02	24
<i>MLLT1</i>	Super elongation complex subunit	6.36E-06		-0.54	2.96E-02	38
Top network genes (ordered by GS)						
<i>TPPI</i>	Tripeptidyl peptidase 1	1.91E-02		0.69	2.85E-03	30
<i>TKT</i>	Transketolase	7.99E-05		0.69	3.27E-03	3
Top hub genes (ordered by k.in)						
<i>RBM17</i>	RNA binding motif protein 17	3.67E-04		0.64	7.70E-03	20
<i>CAPZB</i>	Capping actin protein of muscle Z-line beta	2.64E-05		0.51	4.44E-02	1
<i>YWHAZ</i>	Tyrosine 3-monooxygenase	1.78E-02		0.47	6.43E-02	2
<i>TKT</i>	Transketolase	7.99E-05		0.69	3.27E-03	3

r Pearson correlation between gene expression profile and T1D trait, K.in intramodule connectivity in the green module

Table 2

The results of GO enrichment analysis

GO ID	Term	Gene	Fold enrichment	P-value
GO:0050776	Regulation of immune response	<i>ITGB2, RAC2, CDC37, PTPN11, SKAP2, PSMD13, ITGB1, CD36, PSMF1, CMTM3, TAB2</i>	5.22	4.48E-02

Fold enrichment the score of genes that enriched in the regulation of immune response pathway