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Research Article

Integrated Immune Regulatory Mechanisms Underlying Autoimmune Thyroid Disease

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Abstract:

Autoimmune thyroid disease (AITD) results from various elements which cause immune system tolerance failure and innate immune system activation and improper immune system signal transmission. Research has examined separate elements of these mechanisms, but scientists have not fully grasped how these components work together through genetic and functional processes. This study investigated how specific genes which control immune tolerance and innate immunity and cytokine receptor signaling and development affect autoimmune thyroid disease.A targeted gene-centered in silico approach was applied to analyze AIRE, IFNAR1, IFNAR2, IFNGR2, IL10RB, TLR7, and TBX1. Functional interaction networks were constructed using GeneMANIA and STRING databases. Functional enrichment analyses were performed using Gene Ontology, KEGG, and Reactome pathway databases. Tissue-specific gene expression patterns were assessed using bulk RNA sequencing data from the GTEx database. Network analysis revealed a modular organization, with cytokine receptor–related genes forming a densely connected signaling core, while AIRE and TBX1 occupied distinct subnetworks reflecting immune tolerance and developmental roles, respectively. Functional enrichment analysis highlighted pathways related to immune regulation, innate immune response, and cytokine-mediated signaling. Tissue expression analysis demonstrated widespread expression of receptor genes with variable tissue-specific distribution, supporting receptor-level modulation of immune responsiveness. This research evidence supports a complex system which explains autoimmune thyroid disease through the interaction of immune tolerance systems and innate immune perception and receptor-based immune responses which develop during early life stages. The proposed framework enables scientists to study autoimmune thyroid disease immune system imbalance while helping them create new laboratory tests and medical trial designs.

1. Introduction

Autoimmune thyroiditis (AIT) develops when autoimmune reactions attack thyrocytes which cause thyroid hormone production to become abnormal [1]. AIT is more common in women, and the aetiology is multifactorial. The breakdown of thyroid cells leads to thyroid hormone release into blood which produces hyperthyroid symptoms. The loss of thyroid cells results in euthyroidism for patients before they develop chronic hypothyroidism [1-3].Autoimmune thyroid disease (AITD) appears as a common medical condition which affects patients with autoimmune polyglandular syndromes

(APS) who have APS type 1 or type 2 but APS type 2 patients develop it more frequently [4,5]. The AIRE gene mutation which occurs in APS type 1 leads to the development of this condition because it controls thymic expression of organ-specific autoantigens during immune system development [6,7]. AIRE gene mutations that reduce its function led to negative selection failure which results in thymic deletion of autoreactive T cells that later cause organ-specific autoimmune diseases [6,7].The thyroid gland shows the highest sensitivity to IFN-α immune effects which result in non-autoimmune interferon-induced thyroiditis that primarily affect patients [8]. Research using experimental methods demonstrated that IFN-α affects thyroid-specific genes through its ability to block TSH triggered TG and TPO and NIS gene transcription in human thyrocyte cell cultures. The study investigated how IFN-α affects thyroid gene expression through time-dependent mechanisms which eventually lead to thyroid follicular cell death in experimental models [9-14]. The direct cell-killing effects and gene expression changes occur because of signaling that happens through the type I interferon receptor subunits IFNAR1 and IFNAR2 which exist throughout various body tissues including thyroid tissue. The IFN-α–IFNAR signaling pathway allows the innate immune system to trigger activation which results in thyroid cell destruction that causes thyroid problems during interferon therapy without leading to autoimmune disease development thus showing its role in thyroid immune system disorders [9,14]. The process of organ development includes TBX1 as a developmental regulator which helps create the thymus and thyroid organs while immune system development changes during this period might lead to future immune system problems [15].The peripheral immune responses in autoimmune thyroid disease need two essential elements which consist of innate immune sensing and cytokine-driven signaling [16]. The innate immune cells contain Toll-like receptor 7 (TLR7) which functions as a receptor that detects nucleic acids to trigger autoimmunity through excessive immune system activation and interferon type I production [17]. The immune system depends on three interferon receptor subunits (IFNAR1, IFNAR2 and IFNGR2) and the IL10RB regulatory receptor component to execute cytokine signaling which controls immune system activation and regulation and inflammatory responses. The immune system produces excessive responses through these receptor-based pathways which result in ongoing thyroid inflammation [18,19].Scientists have studied separate components of immune tolerance and innate immune activation and cytokine signaling but they have not created a full system which connects these pathways through genetic relationships and environmental elements of the immune system. The literature lacks thorough investigation about how these genes work together in their molecular connections and their initial tissue and immune cell distribution which could help explain their involvement in autoimmune thyroid disease.The research employed a particular gene-based computational approach to study the combined operation of AIRE and IFNAR1 and IFNAR2 and IFNGR2 and IL10RB and TLR7 and TBX1 genes which control immune tolerance and innate immunity and immune regulation in autoimmune thyroid disease. Our research combined network-based interaction analysis with functional enrichment profiling and tissue-specific expression assessment and immune cell-specific expression analysis to understand how central tolerance mechanisms and innate immune sensing and cytokine receptor signaling pathways create immune dysregulation in autoimmune thyroid disease.

2. Material and Methods

2.1 Study Design and Gene Dataset Collection

This research used gene-centered computational immunogenetic analysis to study immune tolerance and innate immune sensing and immune regulatory pathway genes which contribute to autoimmune thyroid disease. The research team conducted all their analyses through web-based bioinformatics platforms which they used without charge. We chose seven immune-related genes (AIRE, IFNAR1, IFNAR2, IFNGR2, IL10RB, TLR7 and TBX1) which have proven functions in central immune tolerance and innate immune signaling and cytokine receptor-mediated immune regulation and thymic and thyroid development. The Online Mendelian Inheritance in Man (OMIM) database and National Center for Biotechnology Information (NCBI) Entrez Gene database provided gene-level information which included official gene symbols and functional annotations and protein references. The UniProt database provided protein sequence and annotation information which served as input for all following research steps.

2.2 Gene Selection and Gene Interaction Analysis

The chosen genes consist of immune system components which work together to control autoimmune thyroid disease. The researchers included AIRE because it functions as a key component for thymic negative selection and immune tolerance processes. The research studied TLR7 because it serves as the main innate immune receptor which recognizes nucleic acids to trigger

immune system activation. The research analyzed four cytokine receptor subunits which include IFNAR1 and IFNAR2 and IFNGR2 and IL10RB to study their role in immune system signaling and regulatory networks. The researchers included TBX1 because it plays an essential role in thymic and thyroid organ development and because it links to immune system disorders that affect multiple body systems.Network-Based Functional Interaction Analysis Functional interaction networks among the selected genes were analyzed using the GeneMANIA platform (https://genemania.org/). The tool GeneMANIA analyzed the target gene set to discover more genes which share functional connections through co-expression and physical interactions and shared pathways and predicted functional relationships. To validate and complement the GeneMANIA findings, the STRING database (https://string-db.org/) was used as an independent interaction resource. STRING unites experimentally confirmed interactions with curated pathway data and co-expression information and computational models for prediction. The researchers used interaction confidence scores to determine how stable the detected gene–gene relationships were.

2.3 Functional Enrichment Analysis

The research team performed functional enrichment analysis to identify which biological processes and signaling pathways showed the highest activity through the selected genes and their corresponding partner genes. The research team performed enrichment analysis through their examination of data which they obtained from their curated annotation databases. These were Gene Ontology (GO), Biological Process Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome Pathway Database. The system used adjusted p-values to perform multiple testing correction for ranking enriched terms. The analysis focused on immune-related biological processes together with immune regulatory pathways and developmental pathways which affect both immune tolerance and thyroid function.

2.4 In silico Gene Expression Analysis

Tissue-specific expression of IFNAR1, IFNAR2, IFNGR2, and IL10RB was analyzed using publicly available bulk RNA-sequencing data from the GTEx Portal (Analysis Release V10; https://gtexportal.org). Gene expression levels were obtained as transcripts per million (TPM) and evaluated across major human tissues. Expression distributions were visualized using violin plots to illustrate tissue-dependent variability and median expression levels. This analysis was performed as a descriptive in silico assessment of baseline interferon receptor expression without additional stratification or statistical testing.

3. Results and Discussions

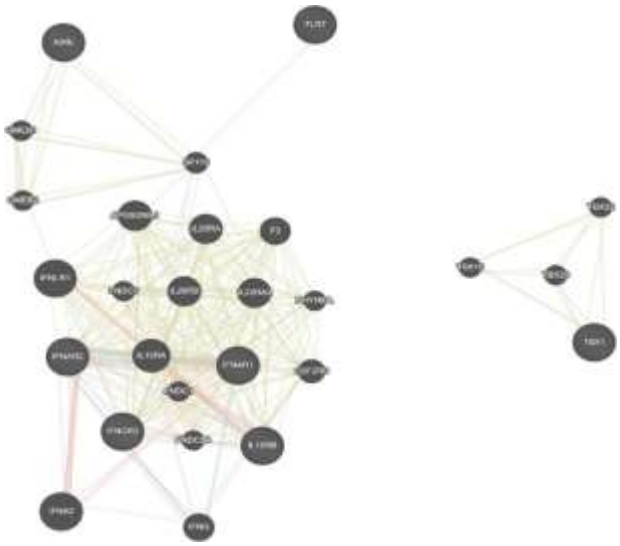


Figure 1. *Functional interaction network of immune tolerance, innate immune, and immune regulatory genes*

Figure 1 illustrates the functional interaction network of the selected genes involved in immune tolerance, innate immune sensing, and immune regulatory signaling. The network demonstrates a densely connected signaling core formed by cytokine receptor–related genes, while AIRE and TBX1 occupy distinct modules reflecting their specialized roles in central immune tolerance and developmental regulation, respectively.Table 1 summarizes the network position and functional relevance of the selected genes within the interaction network. The table highlights the distinction between central signaling nodes, peripheral innate immune components, and developmentally associated genes, emphasizing the multifactorial nature of immune dysregulation in autoimmune thyroid disease.Figure 2 depicts the functional enrichment network of the selected genes, illustrating their associations with enriched biological processes, pathways, and phenotype-related terms. Cytokine receptor–mediated signaling and cellular responses to cytokine stimuli form the central components of the network, with IFNAR1, IFNAR2, and IFNGR2 acting as key connecting nodes. TLR7 is linked to innate immune signaling and altered immune response phenotypes, while IL10RB is associated with regulatory processes. Overall, the network highlights the convergence of immune regulatory and innate immune pathways relevant to autoimmune thyroid disease.

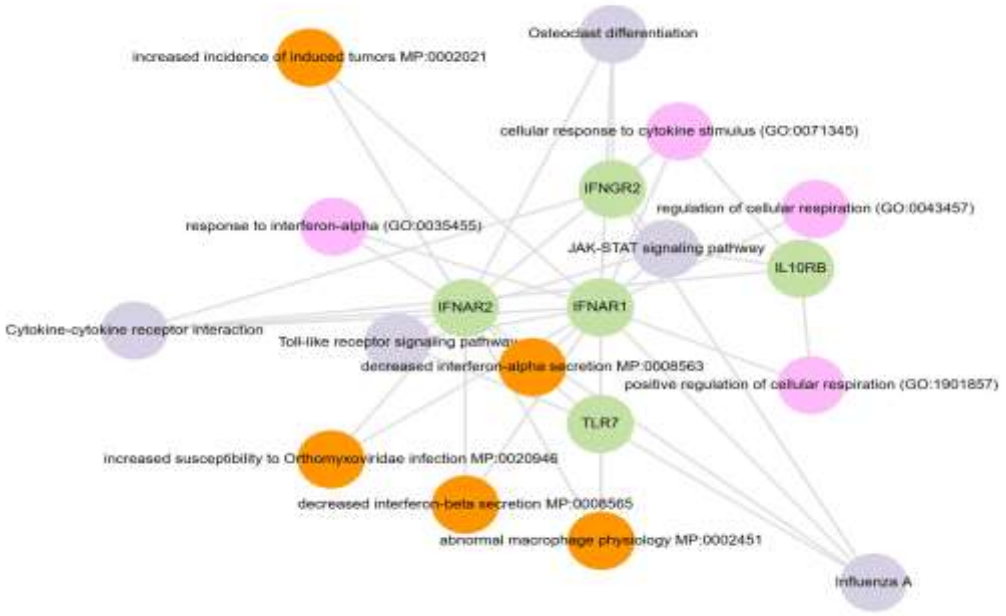


Figure 2. *Functional enrichment network of selected genes*

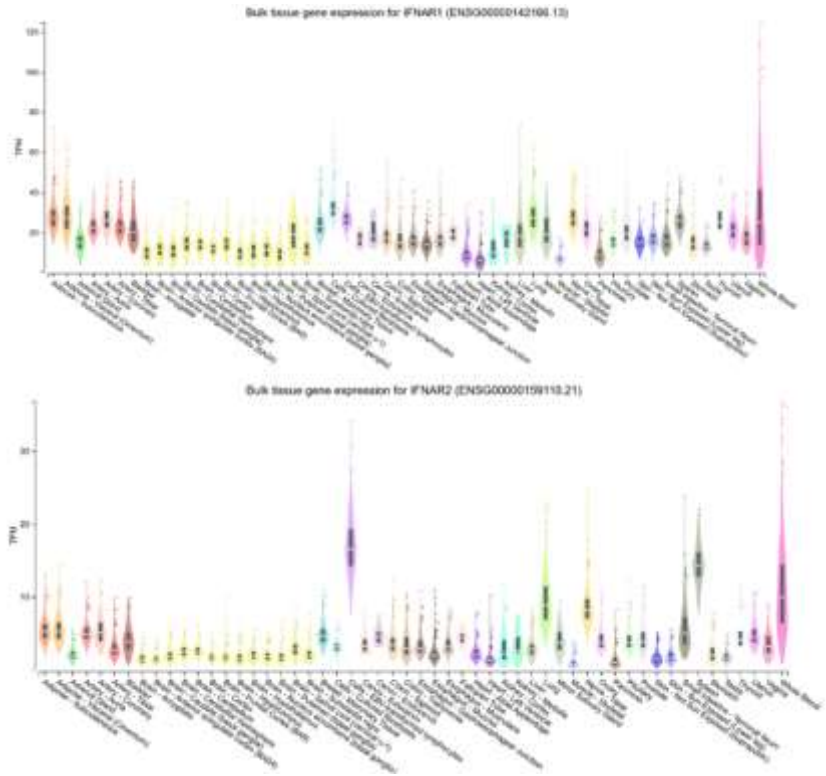


Figure 3. *Tissue-specific expression patterns of IFNAR1 and IFNAR2 across human tissues*

Table 1. *Network characteristics and functional relevance of selected genes*

Gene	Network Position	Primary Functional Role	Key Interpretation
AIRE	Immune tolerance subnetwork	Central immune tolerance and thymic negative selection	Core regulator of self-tolerance mechanisms implicated in autoimmunity
IFNAR1	Central signaling module	Cytokine receptor–mediated immune signaling	Part of a coordinated immune signaling infrastructure
IFNAR2	Central signaling module	Cytokine receptor–mediated immune signaling	Functions together with IFNAR1 in immune regulation
IFNGR2	Central connector node	Immune activation signaling	Reflects receptor-level immune responsiveness

IL10RB	Signaling hub	Immune regulation and anti-inflammatory balance	Integrates regulatory and activation signals
TLR7	Peripheral node	Innate immune sensing	Acts as an upstream immune sensor rather than a central signaling hub
TBX1	Developmental subnetwork	Thymic and thyroid development	Highlights developmental contributions to immune dysregulation

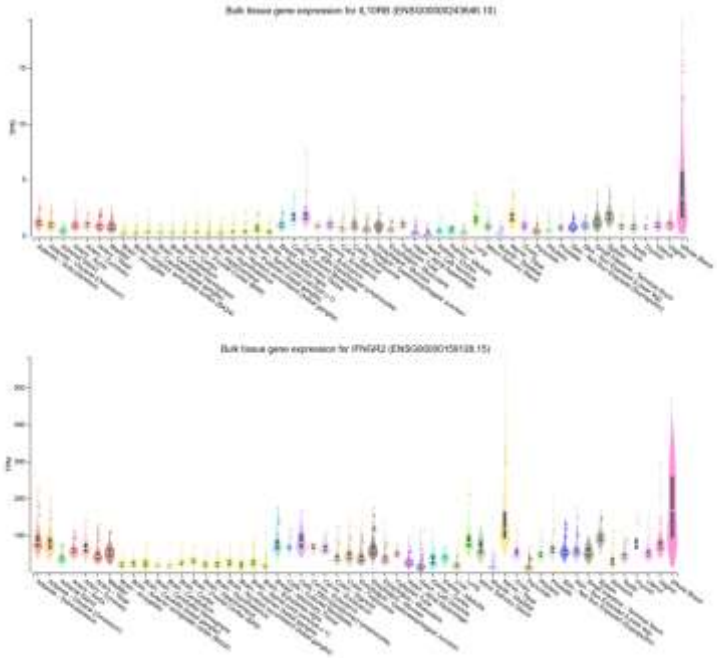


Figure 4. *Tissue-specific expression patterns of IL10RB and IFNGR2 across human tissues*

As shown in Figure 3, IFNAR1 exhibits broad and relatively uniform expression across most human tissues, supporting its role as a constitutive component of type I interferon signaling. In contrast, IFNAR2 displays lower overall expression levels with pronounced variability among tissues, with higher expression observed in immune-related compartments such as whole blood. This differential expression pattern suggests a functional asymmetry within the IFNAR receptor complex, whereby IFNAR1 provides widespread signaling availability, while IFNAR2 may contribute to tissue-specific modulation of type I interferon responsiveness.

Bulk tissue gene expression profiles of IL10RB and IFNGR2 across major human tissues based on the Genotype-Tissue Expression (GTEx) dataset. Violin plots depict the distribution of transcript per million (TPM) values for each tissue. IL10RB demonstrates low but widespread expression across tissues, consistent with its role as a shared regulatory receptor subunit in immunomodulatory cytokine signaling. In contrast, IFNGR2 shows markedly higher expression levels, particularly in immune-related tissues such as whole blood and spleen, reflecting the central role of IFN-γ signaling in cellular immune responses.

AITD develops as a complex condition which results from the combination of immune system tolerance breakdown and innate immune system activation and cytokine signal transmission and targeted tissue susceptibility. The research used a specific gene-focused computational method to analyze the operational links between essential genes which control these biological pathways. The research unites three analytical methods to develop a single framework which links immune tolerance to innate immune sensing and receptor-mediated immune regulation in AITD.

Network-based interaction analysis revealed a modular organization of the selected genes. The cytokine receptor–related genes IFNAR1 and IFNAR2 and IFNGR2 and IL10RB genes form a tightly connected signaling network which shows they operate as a single system to manage immune signaling instead of functioning separately (Figure 1, Table 1). The proteins AIRE and TBX1 operated through separate signaling mechanisms because they established fundamental immune tolerance and they developed the thymus and thyroid organs. The immune system separates into distinct compartments which support the theory that AITD immune dysregulation results from multiple pathways which unite through regulatory and signaling and developmental processes.

The results from functional enrichment analysis confirmed the integrated model that we proposed.

The majority of enriched biological processes and pathways in the study related to cytokine-mediated signaling and cellular responses to immune stimuli and innate immune activation and regulatory processes (Figure 2). The prominence of receptor-level signaling pathways highlights the importance of immune responsiveness and signaling balance in thyroid autoimmunity, rather than excessive activation of a single cytokine axis. The research shows TLR7 operates as the first immune detector which triggers innate immune system activation to generate immune responses in people who have particular genetic mutations.

We performed tissue-specific expression analysis to link their findings with biological processes. The protein IFNAR1 maintained uniform distribution throughout tissues because it serves as a constant component of type I interferon signaling pathways. The expression levels of IFNAR2 showed lower and more unpredictable patterns which reached their highest points in immune-related tissues (Figure 3). The IL10RB gene maintained low expression levels throughout all tissues because it functions as a regulatory protein which controls multiple cytokine signaling pathways. The IFNGR2 gene expression levels increased in blood and spleen tissues because this gene serves as a critical component for cellular immune responses (Figure 4). The studied expression patterns show that AITD immune responses depend on receptor availability which differs between various tissues and their cell populations.

The research study focused on understanding interferon activation of gene expression and immune cell responses to particular triggers. The research investigated three main areas which included receptor-level signaling infrastructure and immune tolerance mechanisms and innate immune sensing systems. The distinction between these two groups of genes remains essential because AIRE and TBX1 function without needing cytokine-induced gene expression to work. The research focuses on immune dysregulation mechanisms in autoimmune thyroid disease through its analysis of specific immune pathways which avoids overextension of any single pathway.

Previous research has built evidence which demonstrates that autoimmune thyroid disease develops through the combination of immune system tolerance breakdown and innate immune system activation and cytokine receptor signaling pathways instead of following a single disease mechanism. The central immune system fails to develop proper tolerance because of AIRE gene mutations which leads to autoimmune polyglandular syndromes and results in autoimmune thyroiditis through the failure of thymic cells to eliminate self-reactive T cells [6,7]. The innate immune system detects thyroid antigens through TLR7 and other Toll-like receptors which activate excessive immune responses and cytokine secretion [17,18]. The immune system depends on IFNAR1, IFNAR2, IFNGR2 and IL10RB cytokine receptor subunits to generate immune responses and maintain regulatory equilibrium because these proteins exist in various parts of the body [8–14,19]. The essential developmental regulators TBX1 which control thymic and thyroid organ development have been linked to autoimmune thyroid disease syndromes which demonstrate how early developmental issues can lead to future immune system problems [15,16]. The research findings support our network- and enrichment-based results which demonstrate that autoimmune thyroid disease develops through multiple factors which work together to regulate immune responses and innate immunity and developmental processes.

The research contains multiple restrictions which need to be recognized. The research used computational methods to analyze public data but it did not conduct experimental or clinical testing to validate its results. The present bulk tissue expression data do not demonstrate how different cell types are distributed across thyroid tissue and immune system areas. The research achieves its best results through its hypothesis-based method which combines immune tolerance with innate immune activation and immune regulatory signaling into a unified systematic model.

In conclusion, our findings support a model in which autoimmune thyroid disease results from the interaction of central immune tolerance defects, innate immune sensing pathways, and receptor-mediated immune signaling, shaped further by developmental factors affecting the thymus and thyroid. This integrative perspective may help refine future experimental and clinical studies aimed at understanding immune dysregulation in autoimmune thyroid disease.

4. Conclusions

This study shows how immune tolerance systems function through innate immune receptors and cytokine receptor signaling pathways which lead to autoimmune thyroid disease development. The research on specific genes demonstrates that immune system dysfunction results from multiple factors which affect both regulatory systems and signaling networks and developmental processes. The research results establish a specific method which scientists can use to study autoimmune thyroid disease through laboratory work and medical testing.

Author Statements:

- **Ethical approval:** TThis study was conducted using publicly available datasets and web-based bioinformatics tools. No human participants, patient samples, or identifiable personal data were directly involved. Therefore, ethical committee approval and informed consent were not required.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
- **Acknowledgement:** The author acknowledges the developers and curators of the publicly available web-based bioinformatics platforms and open-access databases, including GeneMANIA, STRING, Enrichr, OMIM, UniProt, NCBI Gene, and the GTEx Portal, which made this in silico analysis possible.
- **Author contributions:** E.C. and D.K. conceived and designed the study. E.C., B.D., and M.T.O. performed data collection and analysis. D.K. supervised the study and contributed to data interpretation. E.C. drafted the initial manuscript. D.K., B.D., and M.T.O. critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript.
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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