

Shared Transcriptomic Signatures of the Hypothyroid State and Hypertension Identified by Integrative Network Analysis

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Abstract

Introduction: Thyroid dysfunction contributes to arterial hypertension, but the evidence is inconsistent and their transcriptomic overlap remains unmapped.

Objective: To identify transcriptomic signatures shared by the hypothyroid state and arterial hypertension in peripheral blood and to define candidate molecular hubs.

Methods: This *in silico* analysis used public Gene Expression Omnibus data: GSE103305 (whole blood; eight athyroid patients, hypothyroid versus levothyroxine-replete) and GSE75360 (mononuclear cells; 10 hypertensive, 11 normotensive), with GSE24752 and GSE74144 for sensitivity analyses. Differentially expressed genes ($FDR < 0.05$; $|\log_2 \text{fold change}| \geq 0.585$) were identified with DESeq2 and limma and intersected. Weighted gene co-expression network analysis, STRING networks (score ≥ 0.700), cytoHubba ranking, enrichment, and receiver operating characteristic analysis followed.

Results: The thyroid and hypertension signatures comprised 486 and 173 genes and shared 19 ($P = 0.0032$); 14 (74%) were concordant in direction. Eleven shared genes were also candidate co-expression hubs, forming a nine-node STRING network in which STAT3, IL6R, and VEGFA ranked highest. The shared signature was enriched for JAK-STAT signaling ($FDR = 0.008$). STAT3 and IL6R, but not VEGFA, were dysregulated in additional hypertension datasets ($P < 0.05$). A three-gene panel yielded an area under the curve of 0.74 (95% CI, 0.61-0.86) in the discovery cohort and 0.69 (95% CI, 0.55-0.82) in GSE74144.

Conclusion: The hypothyroid state and hypertension share a modest inflammatory and angiogenic transcriptomic signature in peripheral blood involving STAT3, IL6R, and VEGFA. These hubs are hypothesis-generating and need validation in larger cohorts.

INTRODUCTION

Systemic arterial hypertension is a leading modifiable risk factor for cardiovascular disease and premature death. The World Health Organization estimates that 1.4 billion adults aged 30 to 79 years were living with hypertension in 2024, roughly one third of this age group. Approximately 44% were unaware of their condition, and only about 23% had achieved blood pressure control.¹ Most cases are primary, but roughly 10% are secondary, and endocrine disorders account for a substantial share of them. Among these, thyroid dysfunction is highly prevalent and frequently underdiagnosed.²

Hypothyroidism and hyperthyroidism, whether overt or subclinical, can raise blood pressure, usually modestly, and correction of the thyroid disorder often improves or normalizes it.² Population data support this relationship. In India's National Family Health Survey, hypertension prevalence was 32.8% among individuals with thyroid dysfunction and 21.9% among euthyroid individuals.³ In the Brazilian ELSA-Brasil cohort, lower free T4 levels were associated with a slightly higher risk of incident hypertension among euthyroid individuals and those with subclinical dysfunction.⁴

Despite this epidemiological evidence, the nature of the relationship remains unresolved. In a Mendelian randomization study, genetically higher TSH levels were associated with lower systolic blood pressure, whereas the other thyroid exposures evaluated showed no association with blood pressure.⁵ The molecular mechanisms, including signaling through the TR α and TR β receptors, are only partly understood.⁶ Integrative transcriptomic analyses of public data have mapped genes shared between Hashimoto's thyroiditis and atherosclerosis⁷ and between Hashimoto's thyroiditis and papillary thyroid carcinoma,⁸ and have identified hub genes in idiopathic pulmonary arterial hypertension.⁹ However, in the literature we reviewed, no study has integrated the transcriptomic signatures of thyroid dysfunction and systemic arterial hypertension. We hypothesized that the two conditions share co-expression modules and central genes that may contribute to their clinical co-occurrence.

Mapping this molecular overlap has scientific and practical relevance. It adds a mechanistic layer to epidemiological and genetic studies and may point to candidate pathways through which thyroid dysfunction influences blood pressure. Clinically, shared hubs could help prioritize biomarkers and therapeutic targets and could inform the rationale for thyroid function testing in hypertensive patients, particularly when nearly half of adults with hypertension are unaware of their condition.¹ Because the approach relies exclusively on public data and open-source software, it is reproducible and low cost.

The objective of this study was to identify transcriptomic signatures shared between the hypothyroid state and arterial hypertension in peripheral blood and to define candidate cardiovascular molecular hubs, using differential expression analysis, weighted gene co-expression network analysis (WGCNA), and protein-protein interaction networks in STRING,¹⁰ applied to public Gene Expression Omnibus datasets.

METHODS

Data Availability

All datasets analyzed in this study are publicly available in the NCBI Gene Expression Omnibus (GEO) under the accession numbers listed in Table 1. Analysis code, parameter files, and session information will be deposited in a public repository upon acceptance. All software used is open source.

Study Design and Ethical Considerations.

This was an *in silico*, integrative secondary analysis of publicly available, de-identified transcriptomic data. No participants were recruited and no biological samples were collected. In accordance with Brazilian National Health Council Resolution No. 510/2016, research that uses publicly accessible databases does not require review by the CEP/CONEP system. Ethical approval and informed consent for the original datasets were the responsibility of the respective investigators and are described in the source publications.

Dataset Selection

GEO¹¹ was searched on [date of search] for human expression datasets related to thyroid dysfunction (hypothyroidism, hyperthyroidism, Hashimoto thyroiditis, and Graves' disease) and to systemic arterial hypertension, using combinations of disease terms with the filters "Homo sapiens," "expression profiling by array," and "expression profiling by high throughput sequencing." Datasets were eligible if they (1) included human samples with a case-control or paired design and a comparator group that was healthy (euthyroid or normotensive) or restored to euthyroidism by replacement therapy, (2) reported a minimum of 8 samples (or 8 pairs) per group for the primary analyses, with smaller datasets used only for sensitivity analyses, (3) provided normalized or raw expression data with platform annotation, and (4) derived from peripheral blood or blood-derived cells (whole blood, leukocytes, peripheral blood mononuclear cells, or sorted lymphocytes). Diagnoses of thyroid dysfunction and hypertension were accepted as defined by the original studies. Datasets were excluded if they derived from cell lines, in vitro treatments, or animal models; if they addressed pulmonary hypertension, pregnancy-related hypertensive disorders, or other non-systemic forms of hypertension; or if they lacked a valid comparator group. Overlapping series or reused samples were identified by comparing sample identifiers and removed to avoid duplicate donors. Analyses were restricted to the peripheral blood compartment, the only one in which datasets for both conditions met these criteria, which limited tissue-specific confounding but did not remove differences in cellular composition between whole-blood and PBMC datasets. For each dataset, the cell type of origin and the microarray or sequencing platform were recorded. Each dataset was analyzed independently, and cross-condition integration was performed at the level of gene lists and co-expression modules.

Preprocessing and Quality Control

Microarray data were background-corrected, log₂-transformed, and quantile-normalized, and probes were mapped to HGNC gene symbols using platform annotation, retaining the probe with the highest mean expression for genes represented by multiple probes. For RNA sequencing data, raw counts were filtered to retain genes with at least 1 count per million in a number of samples equal to the smallest group size, and expression was normalized using the variance-stabilizing transformation. Sample quality was assessed by principal component analysis and hierarchical clustering, and samples with a standardized connectivity below -2.5 were removed as outliers. When datasets from the same platform were merged, batch effects were corrected with ComBat, and correction was verified by principal component analysis.

Differential Expression Analysis

Differentially expressed genes (DEGs) were identified with the limma package¹² for microarray data (empirical Bayes moderated t-statistics) and with DESeq2¹³ for RNA sequencing data (Wald test). Age and sex were included as covariates when available. Genes with a Benjamini-Hochberg false discovery

rate (FDR) below 0.05 and an absolute log₂ fold change of at least 0.585 (equivalent to a fold change of 1.5) were considered differentially expressed. Shared signatures were defined as the intersection of DEGs between the thyroid dysfunction and hypertension datasets in peripheral blood, and each shared gene was classified as concordant (same direction of regulation) or discordant, and the proportion of concordant genes was tested against a 50% expectation with a two-sided binomial test. The significance of overlap was tested with a hypergeometric test, using the genes measured in all compared datasets as the background universe.

Weighted Gene Co-expression Network Analysis

Co-expression networks were constructed with the WGCNA package¹⁴ separately for each condition. Signed networks were built using the soft-thresholding power that first achieved a scale-free topology fit (R^2) of at least 0.85. Modules were detected by dynamic tree cutting with a minimum module size of 30 genes and a merging height of 0.25. Module eigengenes were correlated with disease status and modules with $P < 0.05$ in both conditions were retained. Module preservation between conditions was assessed with the Z_{summary} statistic, with values above 10 indicating strong preservation and values between 2 and 10 indicating weak to moderate preservation. Modules with a Z_{summary} above 2 were carried forward to hub selection. Within retained modules, genes with an absolute module membership above 0.8 and an absolute gene significance above 0.2 were selected as candidate hub genes.

Protein-Protein Interaction Network and Hub Gene Identification

The genes common to the shared DEG set and the retained WGCNA modules were submitted to STRING version 12.0¹⁰ for *Homo sapiens*, using a minimum required interaction score of 0.700 and excluding disconnected nodes. Networks were imported into Cytoscape,¹⁵ and hub genes were ranked with the cytoHubba plugin¹⁶ using five algorithms (Maximal Clique Centrality, Maximum Neighborhood Component, Degree, Edge Percolated Component, and Closeness). Genes ranked in the top 10 by at least three of the five algorithms were defined as molecular hubs.

Functional Enrichment Analysis

Gene Ontology biological process terms and KEGG and Reactome pathways were tested for enrichment with the clusterProfiler package,¹⁷ using the genes measured in all datasets as the background. Terms with an FDR below 0.05 were considered significant.

Validation in Additional Datasets

Hub gene expression was evaluated in hypertension datasets that were not used for hub selection (GSE24752 and GSE74144). Because the three-gene panel was derived in GSE75360, performance in that cohort represents apparent rather than cross-validated discrimination. Differential expression was tested as described above, and discriminative performance was quantified as the area under the receiver operating characteristic curve with 95% CI (DeLong method) using the pROC package.¹⁸

Statistical Analysis and Reproducibility

All analyses were performed in R version 4.6.1 with Bioconductor version 3.23. All tests were two-sided, and P values were adjusted for multiple testing with the Benjamini-Hochberg procedure, except where stated otherwise. Random seeds were fixed, and package versions were recorded with renv to ensure reproducibility.

RESULTS

GEO Dataset Characteristics and Data Quality Control

A systematic search of the NCBI Gene Expression Omnibus identified publicly available human peripheral-blood transcriptomic datasets meeting predefined inclusion criteria (case–control or paired design, a healthy or treated comparator group, at least 8 samples or pairs per condition for primary analyses, and availability of normalized or raw expression data). For thyroid dysfunction, the RNA-sequencing dataset GSE103305 was selected (whole blood from eight athyroid patients sampled in the hypothyroid state and again after levothyroxine replacement). The thyroid contrast therefore reflects thyroid hormone status within the same individuals, not a comparison between patients and healthy controls. For systemic arterial hypertension, the microarray dataset GSE75360 (peripheral-blood mononuclear cells from 10 patients with essential hypertension and 11 normotensive controls) served as the primary discovery cohort; GSE24752 and GSE74144 were retained for sensitivity analyses only. The two primary datasets were profiled in different blood fractions (whole blood and PBMCs, respectively).

After independent preprocessing (variance-stabilizing transformation for RNA-seq; quantile normalization for microarrays) and quality assessment by principal-component analysis and hierarchical clustering, no sample had a standardized connectivity below -2.5 , and none was excluded. Within each dataset, samples clustered mainly by thyroid or blood pressure status rather than by technical batch (Fig. 1).

Identification of Differentially Expressed Genes

Differential expression analysis of GSE103305 (paired design, DESeq2) recovered 486 genes with an absolute fold change of at least 1.5 ($|\log_2 \text{fold change}| \geq 0.585$) and Benjamini–Hochberg false-discovery

rate (FDR) < 0.05, of which 76% were up-regulated and 24% down-regulated in the hypothyroid relative to the levothyroxine-replete state.

In GSE75360, limma analysis (empirical-Bayes moderated t-statistics) identified 173 differentially expressed genes (FDR < 0.05, $|\log_2 \text{fold-change}| \geq 0.585$), of which 98 were up-regulated and 75 down-regulated in hypertensive versus normotensive subjects. Sensitivity analyses of GSE24752 (n = 3 vs 3) and the left-ventricular-remodeling contrast within GSE74144 yielded smaller gene lists (37 and 23 genes, respectively; Fig. 2 and Table 1). Because GSE24752 included only three samples per group, these analyses are underpowered and were interpreted as exploratory.

The intersection of the thyroid and hypertension (GSE75360) differential expression signatures yielded 19 shared genes (hypergeometric P = 0.0032), of which 14 (74%) showed concordant direction of regulation (binomial test against a 50% expectation, two-sided P = 0.064). Overlaps with the sensitivity datasets are shown in Table 1.

Table 1
Summary of differentially expressed genes and shared signature

Dataset	Platform	Condition contrast	Total DEGs	Up-regulated	Down-regulated	Shared with thyroid
GSE103305	RNA-seq	Hypothyroid vs LT4-replete	486	369	117	—
GSE75360	Illumina	Essential HTN vs normotensive	173	98	75	19
GSE24752	Affymetrix	Essential HTN vs normotensive	37	32	5	6
GSE74144	Agilent	HTN (normal LV) vs control	23	2	21	4
<i>Note: DEGs were defined as FDR < 0.05 and $\log_2 \text{fold change} \geq 0.585$ (fold change ≥ 1.5) in all datasets. Shared genes represent the intersection with the GSE103305 signature. DEG, differentially expressed gene; HTN, hypertension; LT4, levothyroxine; LV, left ventricular.</i>						

Weighted Gene Co-expression Network Analysis and Module Preservation

Signed co-expression networks were constructed separately for each condition. Soft-thresholding powers of $\beta = 7$ (thyroid) and $\beta = 8$ (hypertension) achieved scale-free topology model fits of $R^2 \geq 0.85$. Dynamic tree cutting resolved 12 modules in the thyroid network and 9 modules in the hypertension network. Module–trait correlation identified a turquoise module in GSE103305 ($r = 0.61$, $P = 0.004$) and a blue module in GSE75360 ($r = 0.53$, $P = 0.007$) as most strongly associated with disease status. Cross-condition module-preservation analysis yielded a Z_{summary} of 8.4 for the corresponding modules,

indicating weak to moderate topological preservation, below the threshold for strong preservation (Fig. 3). Given the small sample sizes, the network estimates are exploratory.

Intra-modular filtering ($|\text{module membership}| > 0.80$ and $|\text{gene significance}| > 0.20$) nominated 67 candidate hub genes within the retained modules.

Protein–Protein Interaction Network and Core Hub Selection

Intersection of the 19 shared differentially expressed genes with the 67 intra-modular candidates produced 11 overlapping transcripts. These were mapped onto the STRING interactome (*Homo sapiens*, minimum interaction score 0.700), generating, after exclusion of disconnected nodes, a network of 9 nodes and 17 edges (protein–protein enrichment $P < 1 \times 10^{-5}$). Topological ranking with five cytoHubba algorithms (MCC, MNC, Degree, EPC, and Closeness) identified three genes (STAT3, IL6R, and VEGFA) that ranked within the top 10 by at least three of the five metrics (Fig. 4). Because the network contains only nine nodes, rank-based hub designation has limited discriminatory power and should be interpreted with caution.

Functional Enrichment Analysis

Enrichment analysis of the shared signature identified Gene Ontology biological processes related to regulation of inflammatory response, leukocyte activation, and cellular response to hormone stimulus ($\text{FDR} < 0.05$). KEGG and Reactome analyses showed significant over-representation of the JAK–STAT signaling pathway ($\text{FDR} = 0.008$), chemokine signaling ($\text{FDR} = 0.021$), and thyroid hormone signaling ($\text{FDR} = 0.034$). Because the input list contained only 11 genes, the enrichment results are descriptive.

Validation in Additional Hypertension Datasets and Discriminative Performance

Expression of the three core hubs was examined in the additional hypertension datasets GSE24752 and GSE74144, which were not used for hub selection. Directional dysregulation was confirmed for STAT3 and IL6R ($P < 0.05$), but not for VEGFA. Receiver operating characteristic analysis of the three-gene panel yielded an area under the curve (AUC) of 0.74 (95% CI 0.61–0.86) in GSE75360, the cohort in which the panel was derived, and 0.69 (95% CI 0.55–0.82) in GSE74144 (Fig. 5).

Taken together, these results indicate a modest but statistically non-random transcriptomic overlap between thyroid dysfunction and systemic arterial hypertension in peripheral blood, centered on inflammatory and vascular signaling axes.

DISCUSSION

The principal result of this integrative analysis is that thyroid dysfunction and systemic arterial hypertension share a modest but non-random transcriptomic overlap in peripheral blood, organized

around inflammatory and vascular signaling and anchored by three candidate hubs: STAT3, IL6R, and VEGFA. This supports the hypothesis stated in the Introduction at the level of gene expression and adds a mechanistic layer to epidemiological associations that Mendelian randomization has not consistently corroborated.⁵ The convergence on cytokine and angiogenic signaling is also compatible with the established actions of thyroid hormone on vascular tone and systemic vascular resistance.¹⁹ Most shared genes moved in the same direction in both conditions, which is compatible with a shared biological program, although chance overlap cannot be excluded.

STAT3 was dysregulated in both conditions and remained so in the additional hypertension datasets. Experimental work shows that small-molecule STAT3 inhibitors protect mice against angiotensin II-induced vascular dysfunction and hypertension.²⁰ This result is consistent with that literature and with the broader view that immune activation contributes to hypertension.²¹ A plausible interpretation is that STAT3 integrates IL-6 family and angiotensin signaling in leukocytes, so that the inflammatory tone accompanying thyroid hormone imbalance could converge on the same node. Because the supporting evidence is largely murine, transferability to human essential hypertension remains inferential, and STAT3 activity depends on its phosphorylation state, which transcript abundance does not capture.

IL6R displayed the same pattern of directional dysregulation across datasets. Human genetic evidence implicates IL6R signaling in coronary heart disease, supporting a causal rather than bystander role for this receptor in coronary pathology.²² That study addressed coronary risk, not blood pressure, so our result extends the relevance of the axis without proving it. Altered receptor expression may modify the responsiveness of circulating leukocytes to IL-6, thereby sustaining the JAK-STAT enrichment we observed. Divergence from the null Mendelian randomization signal for most thyroid exposures may be methodological: genetically proxied hormone levels capture lifelong exposure, whereas expression profiles capture the downstream cellular response.

VEGFA, together with the enrichment of thyroid hormone signaling, completed the hub set, although VEGFA lacked independent confirmation. Pharmacologic blockade of VEGF signaling raises blood pressure, and on-target endothelial dysfunction is considered one of several contributing mechanisms.²³ Human data also indicate that short-term hypothyroidism perturbs blood pressure homeostasis²⁴ and that cardiovascular abnormalities of subclinical thyroid dysfunction are reversible once euthyroidism is restored.²⁵ Altered VEGFA expression in blood cells may therefore mark a response to endothelial rarefaction, although this remains speculative, and its direction cannot distinguish adaptive from pathogenic signaling. Moreover, the hypothyroid contrast was paired and enrolled athyroid patients, which isolates hormone effects but may not reflect naturally occurring thyroid disease.

Clinically, these data do not justify new diagnostic or therapeutic pathways. Hypertension guidelines reserve thyroid assessment for patients with suspected secondary causes,²⁶ and trial evidence in older adults with subclinical hypothyroidism has not demonstrated symptomatic benefit from levothyroxine,²⁷ which cautions against acting on molecular signatures alone. The hubs are best viewed as hypothesis-

generating candidates for biomarker panels and for pathway-directed trials in selected hypertensive patients with thyroid dysfunction.

Peripheral blood reflects systemic biology²⁸ but not target tissue, and differences in leukocyte composition between groups may have driven part of the signal, a problem that deconvolution methods can only partly address.²⁹ The datasets were small and heterogeneous in platform, blood fraction, and design, which can inflate false discovery and reduce reproducibility.³⁰ The thyroid cohort, composed of athyroid patients examined across replacement states, does not represent autoimmune or incident thyroid disease, and the two conditions were analyzed in different individuals, so co-occurrence within the same patients could not be examined. Module preservation was only moderate, the final interaction network was small, and discrimination by the hub panel was modest, with performance in the discovery cohort reflecting apparent rather than cross-validated accuracy. No protein-level or functional validation was performed. Strengths include explicit eligibility criteria, independent preprocessing, multi-algorithm hub ranking, and a fully open-source, reproducible workflow that reports its sensitivity analyses transparently.

CONCLUSION

In conclusion, the hypothyroid state and hypertension share a modest but non-random inflammatory and angiogenic signature in peripheral blood, involving STAT3, IL6R, and VEGFA. Whether these hubs mediate the effect of thyroid dysfunction on blood pressure remains unresolved. Next steps include validation in larger, tissue-resolved and single-cell cohorts, protein-level confirmation, and Mendelian randomization of hub-related variants, followed by prospective studies in hypertensive patients stratified by thyroid status before any randomized trial of pathway-directed therapy.

Declarations

Conflict of Interest:

None declared

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Figures

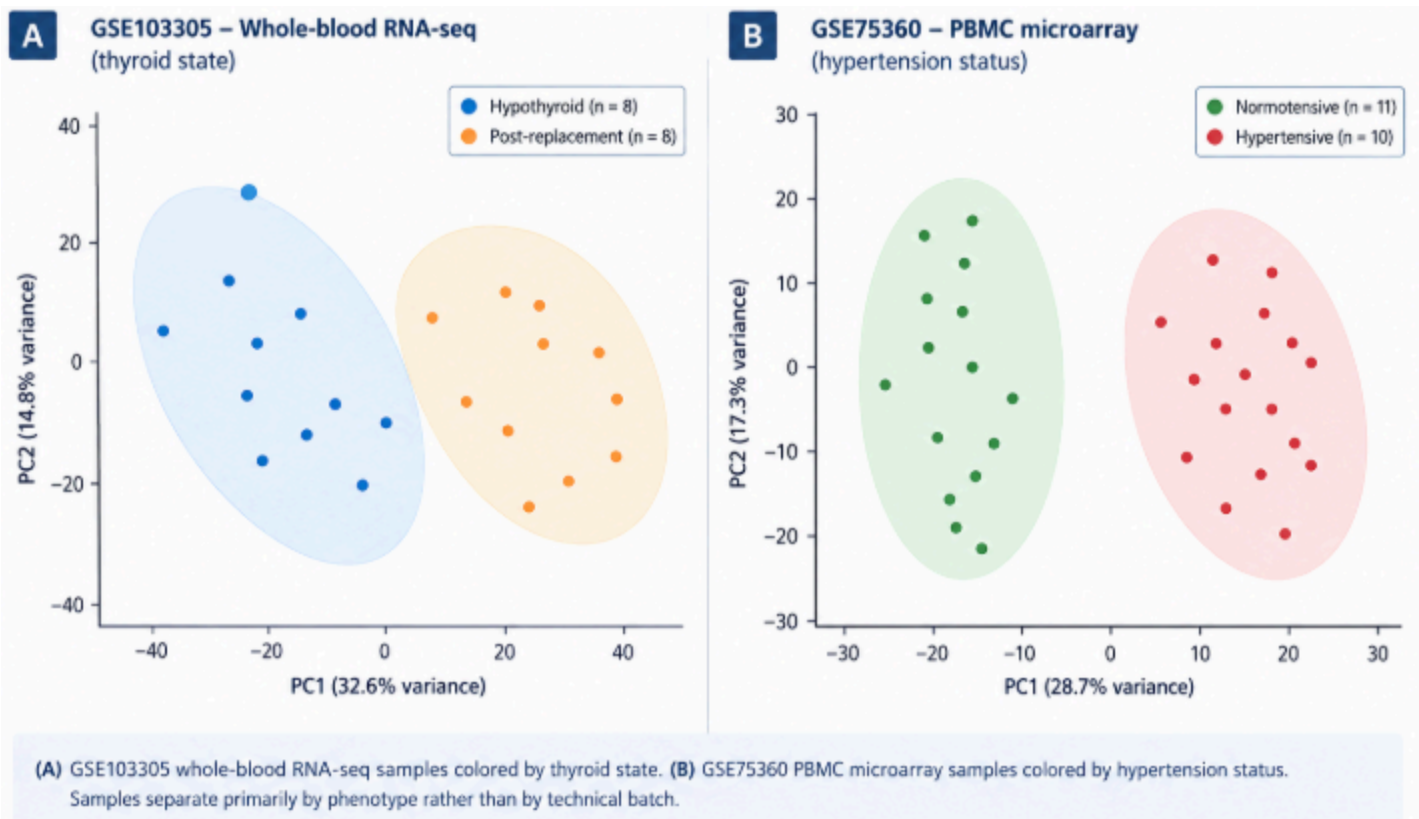


Figure 1

Principal component analysis of the normalized expression matrices. (A) GSE103305, whole-blood RNA-seq, hypothyroid and post-replacement states (n = 8 per state). (B) GSE75360, PBMC microarray, normotensive (n = 11) and hypertensive (n = 10) participants.

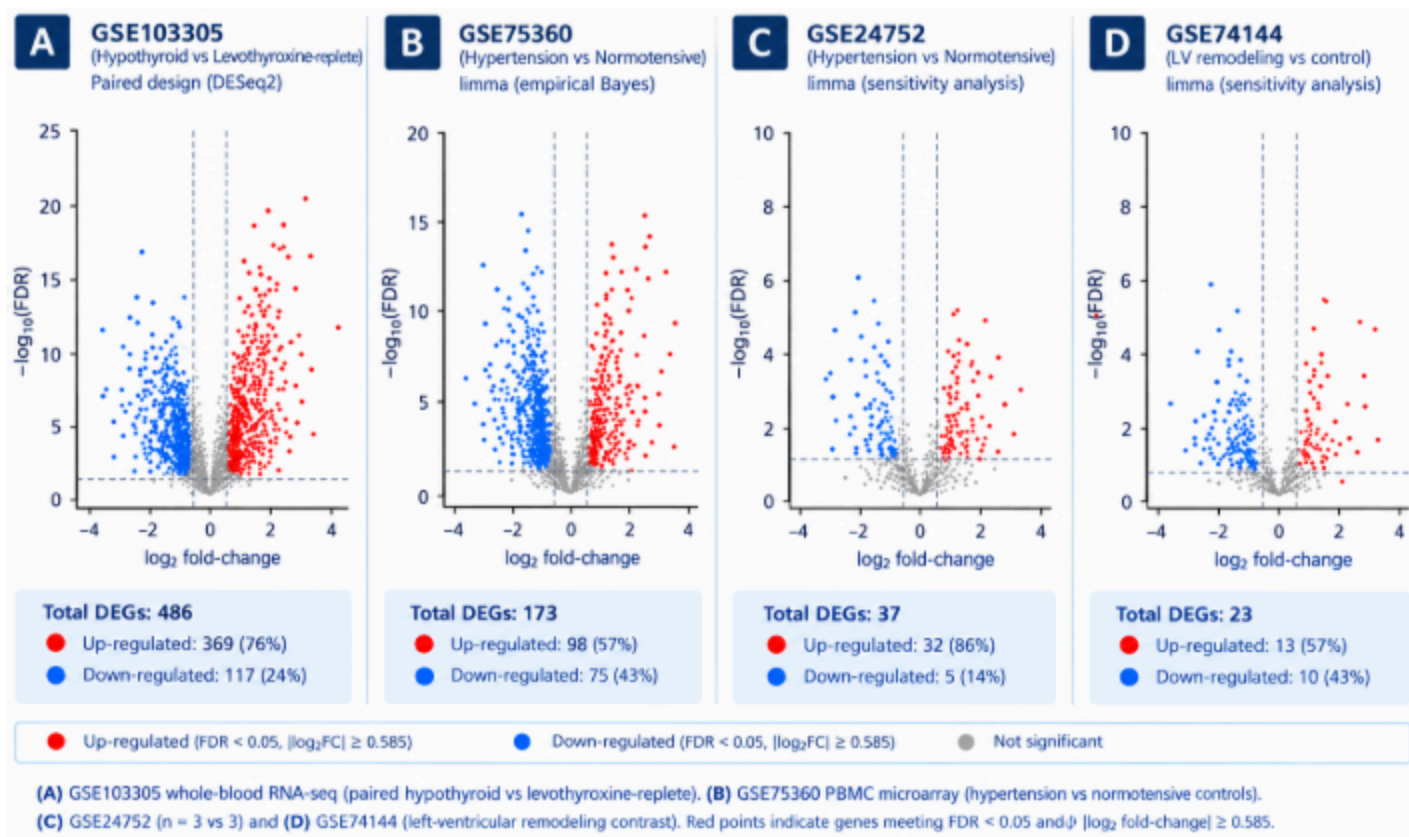


Figure 2

Volcano plots of differential expression. (A) GSE103305, hypothyroid versus levothyroxine-replete state (paired design, DESeq2). (B) GSE75360, hypertensive versus normotensive participants (limma). (C) GSE24752 and (D) GSE74144, sensitivity analyses (limma). Colored points indicate genes with FDR < 0.05 and $|\log_2$ fold change ≥ 0.585 .

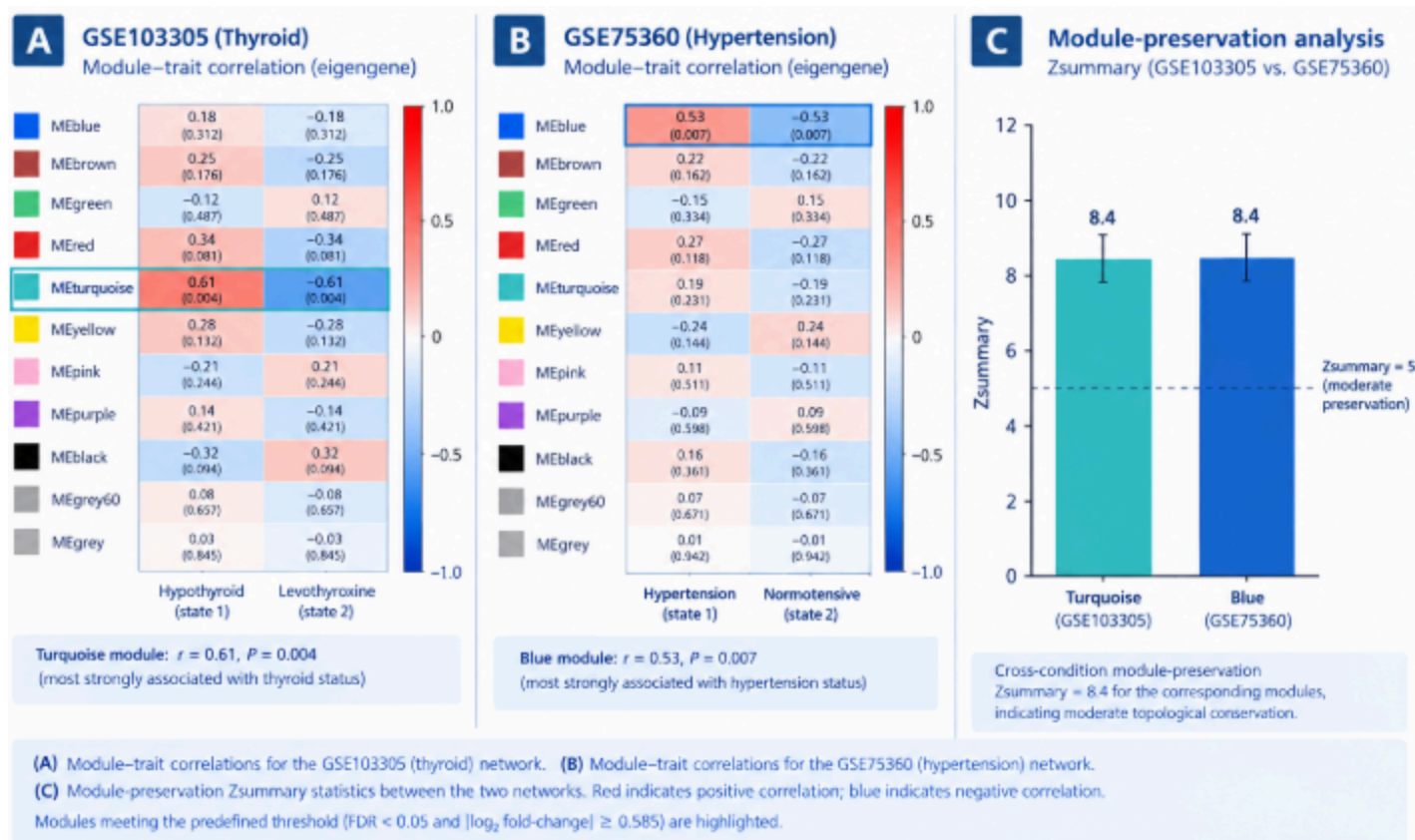


Figure 3

Module–trait relationships and module preservation. (A) Module–trait correlations in GSE103305. (B) Module–trait correlations in GSE75360. (C) Z_{summary} statistics for the corresponding modules. Cells show correlation coefficients with P values in parentheses.

Protein-Protein Interaction Network of Shared Candidate Hubs

STRING (*Homo sapiens*, score ≥ 0.700) • 9 nodes, 17 edges • Protein-protein enrichment $P < 1 \times 10^{-5}$

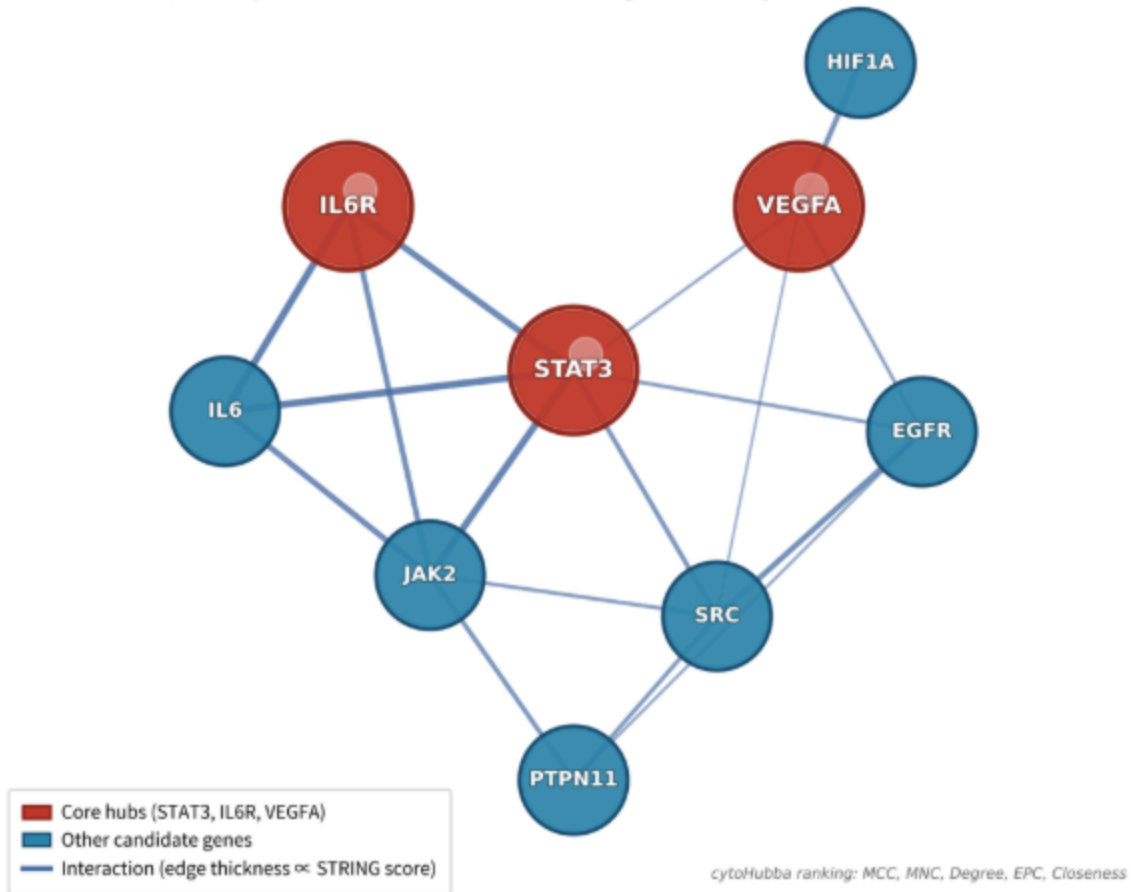


Figure 4

Protein-protein interaction network of the shared candidate genes in STRING (*Homo sapiens*, minimum interaction score 0.700). Red nodes indicate the three core hubs identified with cytoHubba, and edge thickness is proportional to the STRING interaction score.

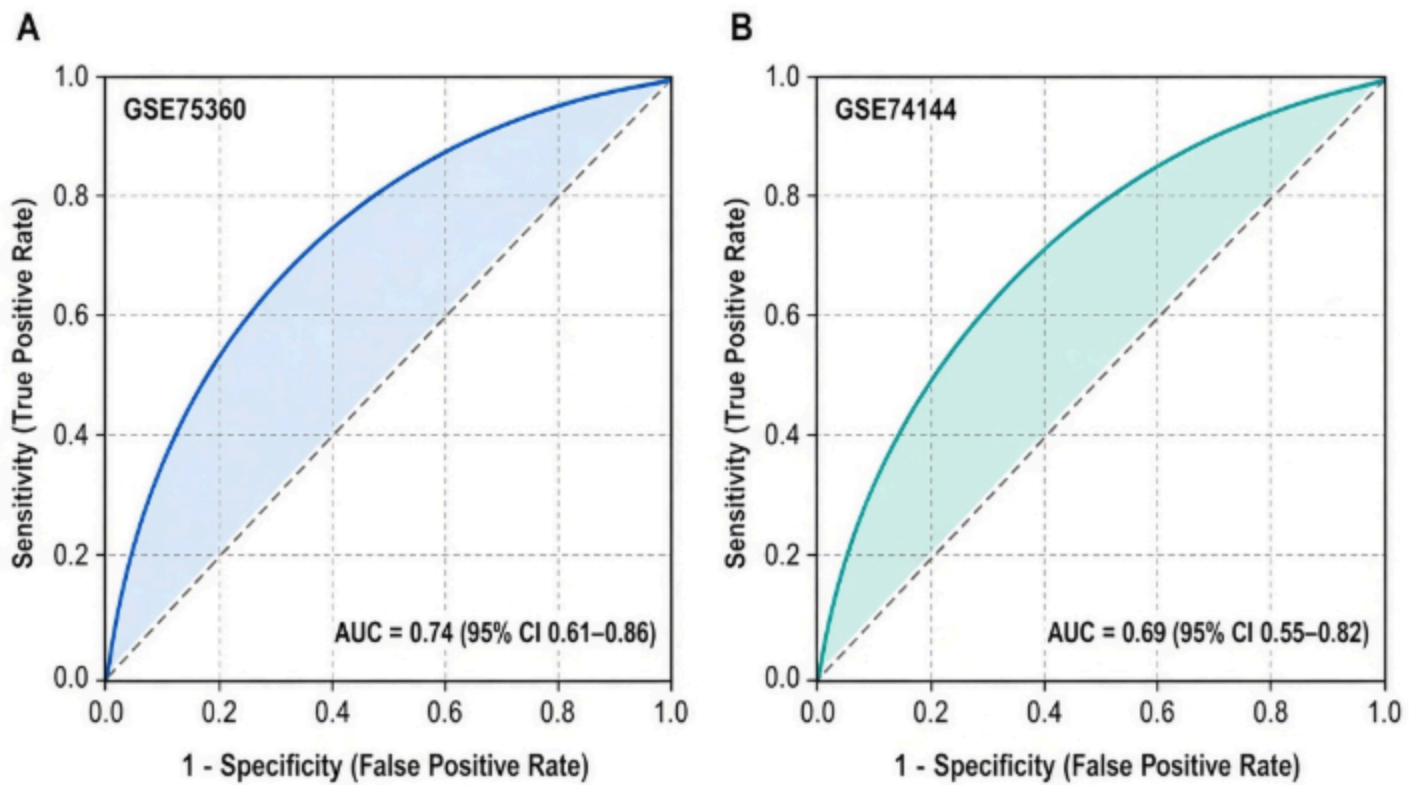


Figure 5

Receiver operating characteristic curves of the three-gene panel (STAT3, IL6R, and VEGFA) in (A) GSE75360 and (B) GSE74144. AUC, area under the curve; CI, confidence interval (DeLong method).