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Thyroid state regulates gene expression in whole blood.

## Thyroid State Regulates Gene Expression in Human Whole Blood

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**Context:** Despite the well-recognized clinical features due to insufficient or excessive thyroid hormone (TH) levels in humans, it is largely unknown which genes are regulated by TH in human tissues.

**Objective:** To study the effect of TH on human gene expression profiles in whole blood, mainly consisting of TR $\alpha$ -expressing cells.

**Methods:** We performed next-generation RNA sequencing on whole blood samples from 8 athyroid patients (4 females) on and after 4 weeks off levothyroxine replacement. Gene expression changes were analyzed through paired differential expression analysis and confirmed in a validation cohort. Weighted gene co-expression network analysis (WGCNA) was applied to identify thyroid state-related networks.

**Results:** We detected 486 differentially expressed (DE) genes (fold-change above 1.5; multiple testing corrected  $P$ -value  $<0.05$ ), of which 76 % were positively and 24 % were negatively regulated. Gene ontology (GO) enrichment analysis revealed that 3 biological processes were significantly overrepresented of which the process translational elongation showed the highest fold enrichment (7.3 fold,  $P=1.8 \times 10^{-6}$ ). WGCNA analysis independently identified various gene clusters that correlated with thyroid state. Further GO-analysis suggested that thyroid state affects platelet function.

**Conclusions:** Changes in thyroid state regulate numerous genes in human whole blood, predominantly TR $\alpha$ -expressing leukocytes. In addition, TH may regulate gene transcripts in platelets.

We performed next-generation RNA sequencing on blood samples from patients on and off levothyroxine. Changes in thyroid state regulate numerous genes and may regulate gene transcripts in platelets.

### Introduction

Thyroid hormone (TH) affects virtually all cells and tissues in the human body. The major biologically active TH is T3 and its genomic actions are mediated by binding to nuclear T3 receptors (TRs) which regulate transcription of target genes (1). Several receptor isoforms are encoded by the *THRA* and *THRB* genes of which TR $\alpha$ 1, TR $\beta$ 1 and TR $\beta$ 2 are the truly T3 binding isoforms (2). The TR isoforms have a distinct expression pattern, with a predominance of TR $\alpha$ 1 in brain, heart and bone and TR $\beta$ 1 in the liver, kidney and thyroid. TR $\beta$ 2 is mainly expressed in

hypothalamus and pituitary and is, thus, involved in the regulation of the hypothalamus-pituitary-thyroid axis (3).

Despite the classical clinical features due to insufficient or excessive TH levels which have been recognized for over 100 years, the underlying molecular mechanisms in humans are not well understood. Knowledge of gene expression modulated by TH is largely derived from animal models or *in vitro* cellular studies used to explore which genes are regulated by TH (4-7). Expanding knowledge of the effects of TH on gene expression in human tissues will provide more insight in the molecular basis of TH action and may lead to a better understanding of the clinical effects of TH in humans.

Progress is limited because most human tissues are not easily accessible. However, blood can be regarded as circulating tissue and contains various cell types including erythrocytes, leukocytes and platelets (8). RNA in whole blood is largely determined by leukocytes. Peripheral blood mononuclear cells (lymphocytes and monocytes) have been shown to mainly express the TR $\alpha$  isoform (9). Therefore, analysis of gene expression in whole blood may potentially be used as proxy for other TR $\alpha$ -expressing tissues. To study the effects of TH on gene expression in human TR $\alpha$ -expressing cells we performed next generation RNA sequencing (RNA-Seq) in whole blood cells from athyroid patients off and on levothyroxine (LT4) treatment.

## Patients and Methods

### *Patients*

Patients were recruited via the outpatient clinic of the Erasmus Medical Center, which is a tertiary referral center for differentiated thyroid cancer (DTC). Patients with DTC undergoing TSH-stimulated <sup>131</sup>I therapy after withdrawal of LT4 were asked to participate in the study. Patients were eligible for inclusion if they had no other malignancies or an active inflammatory disease, were not using any drugs known to influence TH metabolism, and were between 18 and 80 years old. A discovery and a validation cohort were created according to the same protocol.

The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center (MEC 2012-561). Written informed consent was obtained from all study participants.

### *Sample collection and serum analyses*

Peripheral blood samples were obtained from all participants when overt biochemical hypothyroidism was achieved by withdrawal of LT4 substitution in thyroidectomized patients as well as when TSH suppression was achieved after restarting LT4 replacement therapy. Serum free T4 (FT4, reference range 11-25 pmol/l), total T4 (reference range 58-128 nmol/l) and total T3 (reference range 1.4-2.5 nmol/l) concentrations were measured by chemoluminescence assays (Vitros ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Rochester, MI). Serum TSH (reference range 0.4-4.3 mU/l) was measured by an immunometric assay (Immulite 2000 XPI, Siemens, Den Haag, the Netherlands). Whole blood samples were collected in PAXgene tubes. PAXgene tubes contain a proprietary reagent that immediately stabilizes intracellular mRNA, thus reducing mRNA degradation and inhibiting gene induction after phlebotomy. The isolated mRNA represents all blood cells, including polymorph nuclear leukocytes, mononuclear cells, platelets and red blood cells (10).

RNA was isolated using PAXgene blood miRNA kits (PreAnalytiX, Hombrechtikon, Switzerland). For RNA-seq analysis of the discovery cohort, ribosomal RNA and globin mRNAs were first removed from an aliquot of the RNA samples using the Globin-Zero Gold rRNA Removal Kit (Illumina, San Diego, USA) (11). Kapa Stranded RNA library

was prepared (Kapa Stranded RNA kit, Kapabiosystems), followed by sequencing on a HiSeq2500 system, for single end reads, 50 bp in length (Illumina, USA).

### ***Bioinformatic analysis***

We analyzed genes with at least 5 reads in at least 6 samples. The generated sequencing reads were aligned (stranded alignment) against the GRCh38 version of the human reference genome, with RefSeq gene annotation, using Tophat2 (12). Gene counts were generated from the BAM files with HTseq (13). Cufflinks was used to compute transcript abundance estimates in fragments per kilo base of transcript per million mapped reads (FPKMs) (14). Differential expression was calculated with the bioinformatics tool DESeq 2 from Bioconductor, which uses the R statistical programming language (15). Cut off values for significantly expressed genes were a false discovery rate of 0.05 or less and a fold change of 1.5. To visualize the clustering of the samples, principal component analysis (PCA) was performed. The normalized data file was transposed and imported into OmniViz version 6.1.13 (Instem Scientific, Inc., Stone, Staffordshire, UK) for further analysis. The geometric mean of the intensity of all samples was calculated. The level of expression of each gene was determined relative to this geometric mean and  $^2\log$  transformed. The geometric mean of all samples was used to ascribe equal weight to gene expression levels with similar relative distances to the geometric mean. The data were deposited in NCBI/GEO reference (URL) and Accession number GSE103305.

We used functional enrichment analysis to explore the biological significance of DE-genes and of genes in WGCNA-modules (see below) using DAVID (EASE) (16). DAVID calculates significantly overrepresentation of gene ontology (GO)-classified biological processes by comparing the number of genes in a gene list for a biological process to the number of genes for that biological process from the RNA-seq analysis (17). Biological processes are shown which were significantly enriched after correction for multiple testing.

The independent skeletal muscle dataset for comparative analysis consisted of 10 thyroidectomized patients with DTC four weeks after LT4 withdrawal (before TSH stimulated  $^{131}\text{I}$  scintigraphy) and 8 weeks after subsequent LT4 replacement (18).

To study the expression of the TR isoforms in different blood cell types, independent datasets on RNA sequencing in platelets (NCBI SRA SRP028846), lymphocytes (E-MTAB-2319) and neutrophils (GEO Series GSE59528) were collected from previous studies (19-21). FPKM values of TR $\alpha$ 1 and TR $\beta$ 1 in the different cell types were compared using a T-test.

### ***Quantitative PCR (qPCR)***

To confirm the RNA-seq results by an independent technique, qPCR was used to measure expression of selected genes. QPCR was performed on cDNA produced from RNA before applying Globin-Zero Gold rRNA Removal Kits on the discovery cohort and on cDNA from the validation cohort. The primer sequences are presented in Supplemental Table 1. RNA levels are expressed relative to the geometric mean of the house-keeping genes GAPDH and ACTB.

### ***Statistical analysis***

Likelihood and significance of the overlap with DE-genes in muscle samples off and on LT4 treatment previously reported were tested using the  $\chi^2$ -test (18). Thyroid function tests and leukocyte counts were compared off and on LT4 treatment using the Wilcoxon signed rank test. FPKM values of the TR $\alpha$ 1 gene were compared off and on LT4 using a paired T-test. Statistical analysis was performed using SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA).

### ***Weighted gene co-expression network analysis***

We performed a weighted gene co-expression network analysis (WGCNA) to discover co-expressed TH-related networks (modules) (22,23). In short, a weighted adjacency matrix containing pair-wise connection strengths was constructed by using the soft-thresholding approach ( $\beta = 6$ ) on the matrix of pair-wise correlation coefficients. A connectivity measure ( $k$ ) per gene was calculated by summing the connection strengths with other genes. Modules were defined as branches of a hierarchical clustering tree using a dissimilarity measure (1 - topological overlap matrix) (23,24). Each module was subsequently assigned a color. The gene expression profiles of each module were summarized by the module eigengene (defined as the first principal component of the module expression levels). Each module eigengene was correlated to thyroid status (with age, BMI, gender, leukocytes and leukocyte differentiation as covariates) using the WGCNA R package (FDR 5%).

## Results

### *Study population*

We studied whole blood samples from 8 athyroid patients (4 females and 4 males) off and on LT4 treatment. Characteristics of the patients are shown in Supplemental Table 2. Table 1 shows the serum TH concentrations and total leukocyte counts, including leukocyte differentiation, off and on LT4 treatment. As expected, the thyroid function tests were significantly different. Neither total leukocyte counts nor leukocyte differentiation showed significant differences, except for a slight increase in monocyte number during LT4 treatment.

### *Standard gene expression analysis*

To assess the effects of thyroid state on gene expression in whole blood cells, we performed next-generation RNA-seq on samples drawn from patients off and on LT4 replacement therapy. At least 15 million reads were generated for each sample and approximately 95% of these reads were aligned. We first quantified both TR isoforms to confirm that TR $\alpha$  is the main receptor isoform expressed in whole blood samples (Fig. 1A). TR $\alpha$ 1 expression appeared reduced during LT4 therapy compared with the hypothyroid state ( $P=0.008$ ). TR $\alpha$ 1 expression quantified by qPCR analysis also showed a trend towards downregulation, although this failed to reach statistical significance (Supplemental Fig. 1). Re-analysis of the TR isoforms in different blood cell types of independent datasets showed that TR $\alpha$ 1 expression was much higher than TR $\beta$ 1 in platelets, lymphocytes and neutrophils suggesting that TR $\alpha$ 1 is indeed the major T3 receptor isoform expressed in whole blood (Supplemental Fig. 2) (19-21).

After filtering for expressed genes, 16014 genes remained for analysis. PCA analysis showed a high degree of clustering of patients off and on LT4 treatment. We detected 1227 differentially expressed (DE) genes (multiple testing corrected  $P$  value  $< 0.05$ ). We selected 486 DE-genes with an absolute fold-change above 1.5 (Supplemental Table 3), shown as hierarchical clustering in Fig. 1B. This indicates that thyroid state has both positive and negative effects on gene regulation (76 % upregulated; 24 % downregulated). The 47 genes that differed at least 2-fold in expression are presented in Table 2.

### *Validation of selected genes*

To validate the RNA-seq data from the discovery cohort, qPCR was used as an independent technique to measure the relative expression of selected DE genes. Genes were selected based on robust baseline expression, robust fold change in expression and low adjusted  $p$ -value. All upregulated and most downregulated genes showed significant differential expression using qPCR (Fig. 2). We validated our findings in an independent cohort of which the baseline

characteristics are shown in Supplemental Table 4. qPCR analysis of the validation cohort confirmed that all genes were similarly regulated by TH as in the discovery cohort, although this was not significant for some small nucleolar RNAs (snoRNAs) and PDE5A (Fig. 2).

### **GO analysis**

Next, we analyzed whether the 486 DE-genes were associated with specific biological processes. GO enrichment analysis revealed that 7 biological processes were significantly overrepresented after correction for multiple testing (FDR 0.05) (Supplemental Table 5). Because some GO terms overlapped based on similar groups of genes, the number of biological processes was reduced to 3 (Fig. 3).

### **Comparative transcriptome analysis**

To explore to which extent gene expression in whole blood parallels gene expression in other tissues, we investigated the overlap with gene expression in skeletal muscle in different thyroid states (18). This dataset contained 607 DE-genes (fold-change > 1.5) in muscle samples off and on LT4 treatment as previously reported. Comparative transcriptome analysis of the 486 DE-genes (fold-change > 1.5) of the present study with the muscle dataset revealed 26 genes that were shared between both tissues, which is a significant 2.3-fold enrichment ( $P=4.1 \times 10^{-5}$ ) (Supplemental Table 6). Of these 26 shared genes, 6 were regulated in the opposite direction. If removed from the analysis, this resulted in a 1.7-fold enrichment ( $P=0.02$ ).

### **WGCNA**

To further explore TH-dependent transcriptional patterns in the current dataset, we employed WGCNA analysis. WGCNA can reveal the underlying organization of the transcriptome based on co-expression relationships. WGCNA complements traditional DE analyses by providing a system level framework for the understanding of transcriptional profiles. This has been shown particularly helpful in transcriptome analysis of samples composed of distinct cell and tissue types (25).

Therefore, WGCNA is potentially useful to identify specific cell types responsive to different thyroid states. First, the genes showing most variability between samples (i.e. at least a 2.0-fold change in level of expression from the global mean) were selected. This resulted in 6649 genes, which were determined by their coefficient of variance, rather than any sample characteristics such as disease status. Subsequently, unsupervised hierarchical clustering led to the identification of 17 co-expression modules (Fig. 4A). Modules correspond to branches and are color-coded, ranging in size from 67 genes in the light cyan module to 1737 in the turquoise module (Supplemental Table 7). Next, modules were identified that were significantly associated with clinical parameters, including age, sex, leukocytes and thyroid state. Therefore, the summary file (eigengene) for each module was correlated with the clinical parameters to select the most significant associations (Fig. 4B). The strength of WGCNA analysis is well exemplified by the tan module. The unbiased approach identified 120 genes in this module, which were closely linked according to WGCNA. Subsequent inspection learned that this module mainly contained genes expressed from the Y-chromosome. Regression analysis afterwards correctly identified (male) sex to this module ( $r=0.97$ ,  $p=2.0 \times 10^{-10}$ ). Using similar analysis, the blue and the midnight blue modules were significantly associated with thyroid state. The blue module correlated positively with thyroid state ( $r=0.65$ ,  $p=0.007$ ) and contained numerous genes (213 of the 814 genes in this module) that were also found significantly regulated by TH in the DE analysis. The positive correlation reflects that most blue module genes were upregulated by TH.

Next, we performed a GO enrichment analysis for the genes in the blue module (Supplemental Table 5). The biological process hemostasis was significantly enriched in the blue module. Of note, many genes in the blue module appeared transcripts expressed in platelets (e.g. P2RY12, PF4). The module midnight blue was negatively correlated with thyroid state ( $r = -0.78$ ,  $p = 4.0 \times 10^{-4}$ ) and contained mostly genes (32 of the 74 genes) that were regulated by TH as well in the DE analysis, of which 26 (75%) were down-regulated. Many genes in the module midnight blue belonged to the class of snoRNAs and it contained mostly genes without a GO annotation. Thus, WGNCA analysis suggested that gene expression not only in leukocytes but also in platelets is dependent on thyroid state.

## Discussion

In humans, genes that are regulated by thyroid state are largely unknown. Previously, we and others discovered genes that are dependent on thyroid state in human skeletal muscle (18,26). *Ex vivo* studies have identified T3-responsive genes in human skin fibroblasts and adipocytes (26-28). The present study identifies numerous genes in human whole blood samples that are regulated by thyroid state. Similar to previous transcriptome analysis studies, TH largely positively regulates gene expression, although a considerable number of genes is downregulated. As RNA from leukocytes is a major determinant to total RNA in blood (after removal of globin RNA), it is likely that the detected DE-genes largely represent the effects of thyroid state on leukocytes.

To improve the yield of our genome-wide expression profiling, we performed WGCNA and compared these results with those of a standard analysis based on differential expression. While a standard analysis typically discloses lists of DE-genes, it fails to recognize the different connections between them. Indeed, WGCNA mapped many genes related to thyroid state into two large co-expression modules (modules blue and midnight blue). GO enrichment analyses of the TH-associated module blue showed predominance of the biological process hemostasis of which the involved genes were upregulated by TH. Of note, many genes were platelet specific transcripts (e.g. P2RY12, PF4). Because platelets are anucleate, their mRNA content derives from nucleate precursors, which is translated into proteins (40). The product of *P2RY12* is a purinergic G-protein coupled receptor, which plays a crucial role in thrombus formation (29). P2RY12 inhibitors, such as clopidogrel and ticagrelor, have antithrombotic effects and are widely used in patients with acute coronary syndromes and in the secondary prevention of thrombotic events in vascular diseases (30). *PF4* codes for a chemokine (platelet factor 4) which is synthesized in megakaryocytes and stored in platelet alpha granules. When platelets are activated, PF4 is released from the alpha granules facilitating thrombosis (31). The observation that circulating PF4 levels are decreased in patients with subclinical autoimmune hypothyroidism supports our findings (32). *F13A1*, another upregulated gene by TH, encodes the coagulation factor XIIIa subunit. Coagulation factor XIII is important for stabilisation of the fibrin clot. Pietzner *et al.* recently demonstrated that thyrotoxicosis increased the levels of several coagulation cascade proteins, including coagulation factors IX, XI and XIII in plasma (33). Together, these data suggest that TH induces transcription of pro-thrombotic genes. This is in line with the observation that hyperthyroidism increases the risk of thrombosis (34,35). Even high-normal thyroid function within the reference range is associated with stroke, independent of classical cardiovascular risk factors (36). Previously, it has been shown that the production of several coagulation factors produced in the liver is enhanced by TH (37,38). Although we were unable to assess thrombocyte counts, other studies have demonstrated that platelet number is

independent of thyroid state (39). The present study suggests that increased TH levels also positively regulates pro-thrombotic factors in platelets.

The second WGCNA-module that correlated negatively with thyroid state was mainly composed of snoRNAs which were mostly down-regulated by TH. SnoRNAs are classified into two families (box C/D snoRNAs and box H/ACA snoRNAs) and are required for posttranscriptional modifications of ribosomal RNA (rRNA)(41). As the relationship between snoRNAs and TH has not been reported before, further studies are needed to understand the consequences of this finding.

Although the effects of thyroid state on peripheral blood cells (leukocytes, platelets) are of interest in itself (see above), we explored to which extent those findings were relevant for other tissues. As whole blood samples mainly contain TR $\alpha$ -expressing cells, our results putatively reflect the effect of TH on gene expression via TR $\alpha$ . As TR $\alpha$  is abundantly expressed over TR $\beta$  in skeletal muscle, we sought to determine overlap between genes dependent on TH in muscle and genes identified in the present study. The overlap with the DE-genes from our previous study in muscle samples was small but statistical significant. This finding suggests that a subset of genes is commonly regulated by TR $\alpha$ 1 in various tissues. A few of those genes were regulated in the opposite direction. Although counterintuitive, this phenomenon has been described before (e.g. the well-known T3-responsive gene Reelin) (42). However, the lack of a large overlap also indicates that thyroid state affects different genes in different tissues. Together, these findings may limit the use of whole blood samples as a proxy for the effects of TH on other TR $\alpha$ -expressing tissues in humans (18).

Several strengths and limitations are worth mentioning to provide context to our findings. A first limitation is that the observed changes in gene expression may not necessarily reflect direct effects of TH. Gene transcription may also be indirectly dependent on TH if it modulates intermediate signaling molecules. Our studies, thus, reflect the net effect on gene expression upon changes in thyroid state. In this context, it is worth mentioning the somewhat conflicting results on the limited downregulation of TR $\alpha$ 1 in the RNA-seq analysis, which is in line with a previous study that observed a down-regulation of TR $\alpha$ 1 in hematopoietic progenitor cells obtained from the peripheral blood of hyperthyroid patients compared with a euthyroid control group, although qPCR quantification failed to reach statistical significance (43). These observations suggest that the expression of TR $\alpha$ 1 in peripheral blood cells is negatively dependent on thyroid state, possibly dampening the effect size of changes in thyroid state. Second, one should realize that whole blood contains many different cell types. Although WGCNA was relevant in this context, other subtle changes may have gone unrecognized. Third, DE genes in hypothyroid *versus* mild thyreotoxic state (as in this study) are not necessarily the same DE genes as in hyperthyroid *versus* mild thyreotoxic state. Finally, since the patients adhered to an iodine deficient diet at the time of hypothyroidism, direct effects of changes in iodine state on gene expression cannot be excluded. Our study has several strengths. First, the study design included paired analyses, which has the advantage to reduce confounders and variability. Second, the results were confirmed in an independent cohort, substantiating the robustness of our findings. Third, we were able to study extreme differences in thyroid state in human subjects without thyroid autoimmunity.

In conclusion, we demonstrated for the first time that thyroid state regulates numerous genes in human whole blood. Furthermore, we found that thyroid state affects gene transcripts in platelets, which contributes to the understanding of thrombosis in hyperthyroidism. The overlap with previously reported DE-genes in muscle samples indicate that a subset of genes is

commonly regulated inTR $\alpha$ -expressing tissues in humans. Future studies should explore if specific transcripts in whole blood can be useful biomarkers for tissue-specific thyroid state.

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**Figure 1. A.** Gene expression (FPKMs) of TR $\alpha$ 1 and TR $\beta$ 1 off and on levothyroxine (LT4) treatment. \* P <0.05, \*\*P<0.01, \*\*\* P<0.001. **B.** OmniViz Treescape shows the hierarchical clustering of differentially expressed genes off and on LT4 treatment. Red: upregulated genes compared with the geometric mean; blue: downregulated genes compared with the geometric mean. The color intensity correlates with the degree of change.

**Figure 2.** Verification of RNA-seq results by qPCR. 6 upregulated (**A**) and 6 downregulated genes (**B**) were selected from the RNA-seq results. Results are shown as 2log ratio of the fold change in gene expression off and on levothyroxine treatment. \* P <0.05, \*\*P<0.01, \*\*\* P<0.001.

**Figure 3.** GO enrichment analyses of differentially expressed genes off and on LT4 treatment. The biological processes are shown on the Y axis.

**Figure 4. A.** Network construction identifies distinct modules of co-expressed genes. Modules of co-expressed genes were assigned colors corresponding to the branches indicated by the horizontal bar beneath the dendrogram. **B.** Heatmap plot of the adjacencies in the eigengene network including several traits. Each row and column in the heatmap corresponds to one module eigengene (labeled by color) or trait (labeled on the x-axis). In the heatmap, green color represents low adjacency (negative correlation), while red represents high adjacency (positive correlation). The Blue and the Midnight Blue modules were significantly associated with thyroid state.

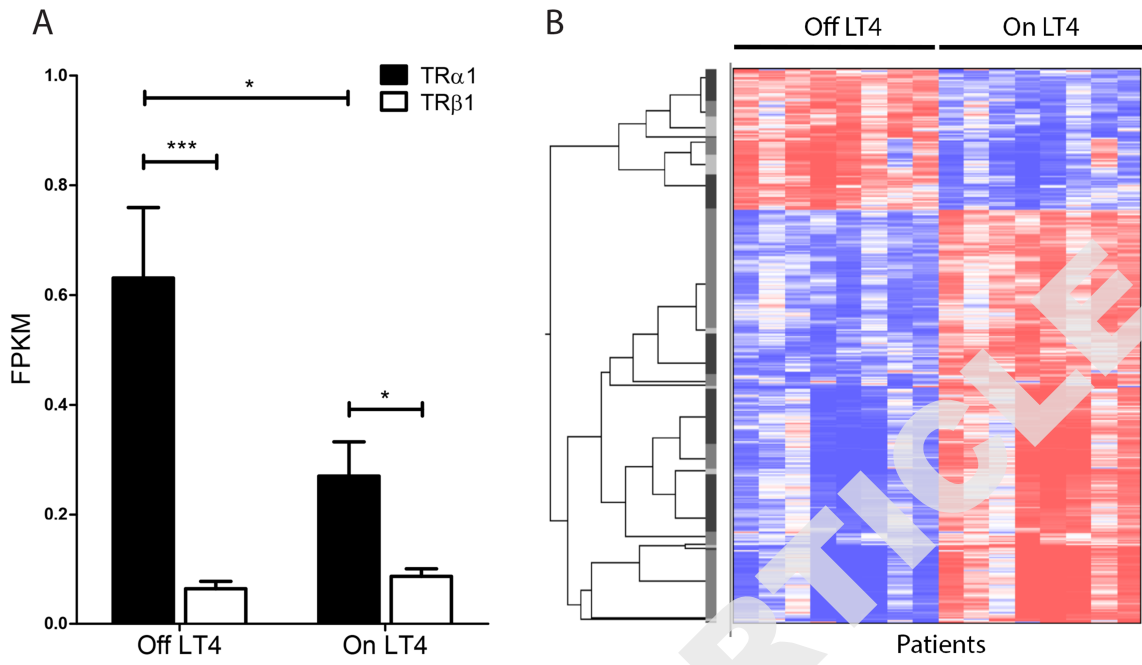
**Table 1.** Thyroid function tests and leukocyte counts

	Off LT4	On LT4	P
TSH (0.4-4.3) (mU/l)	78.0 (48.5-117.5)	0.07 (0.004-0.11)	0.012
Total T3 (1.4-2.5) (nmol/l)	0.72 (0.63-0.83)	2.11 (1.94-2.28)	0.012
Total T4 (58.0-128.0) (nmol/l)	17.0 (14.0-18.0)	155.0 (135.5-170.5)	0.018
Free T4 (11.0-25.0) (pmol/l)	1.55 (1.12-2.17)	26.2 (22.3-29.5)	0.012
Leukocytes (3.5-10.0) (x10 <sup>9</sup> /l)	7.6 (5.5- 8.1)	7.4 (5.5-8.7)	0.26
Neutrophils (40.0-80.0) (%)	60.0 (52.0-67.0)	62.8 (58.2-65.7)	0.50
Lymphocytes (15.0-50.0) (%)	32.4 (25.1-35.9)	27.3 (23.5- 28.6)	0.13
Monocytes (5.0-14.0) (%)	4.7 (4.6- 7.8)	8.1 (6.9-9.6)	0.018
Dosage LT4 ( $\mu$ g/kg)		2.3 (2.0-2.7)	
Time between tests (wks)		22.5 (17.2-24.0)	

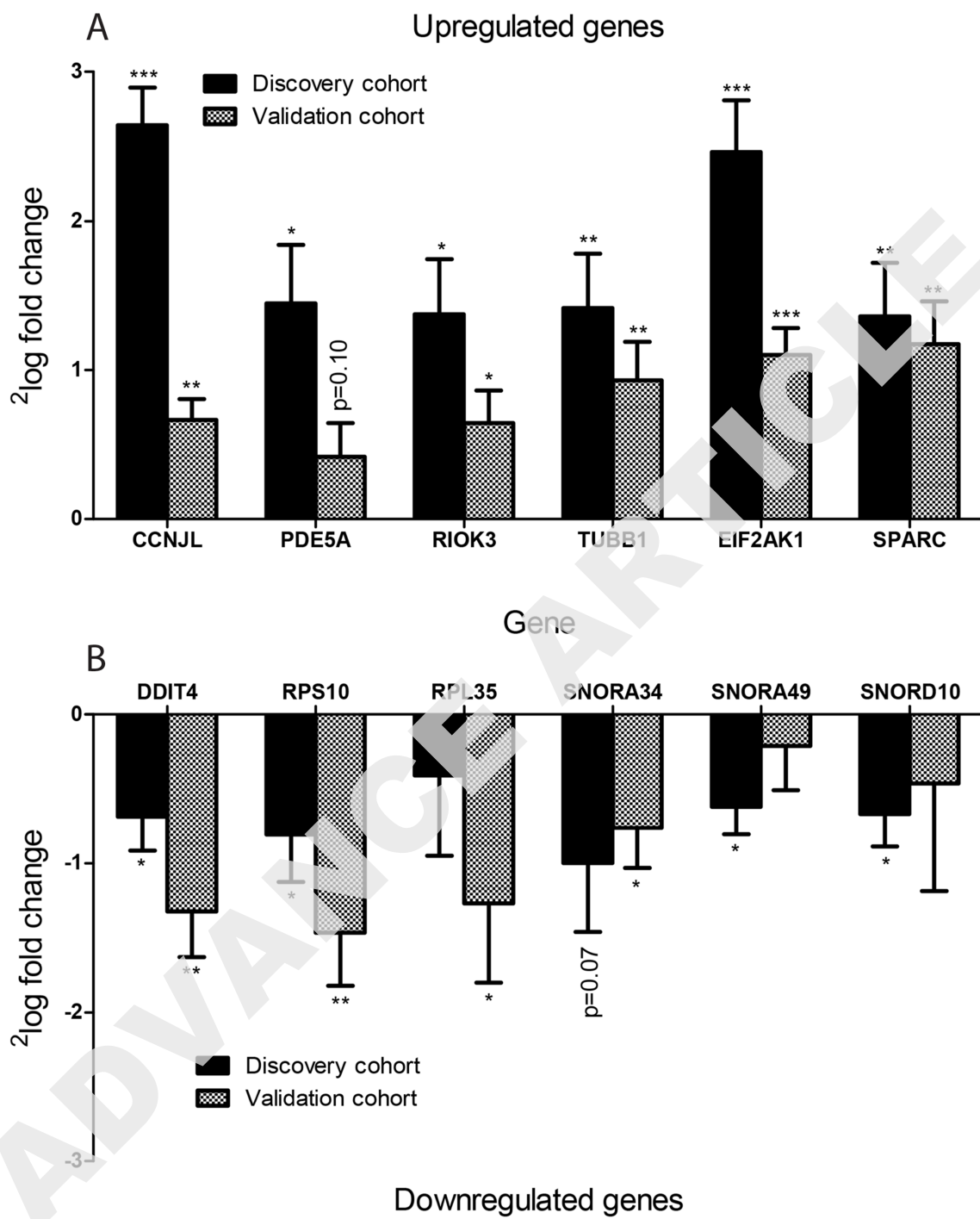
**Table 2.** Genes that differed 2-fold in expression (<sup>2</sup>log ratio >1 or < -1)

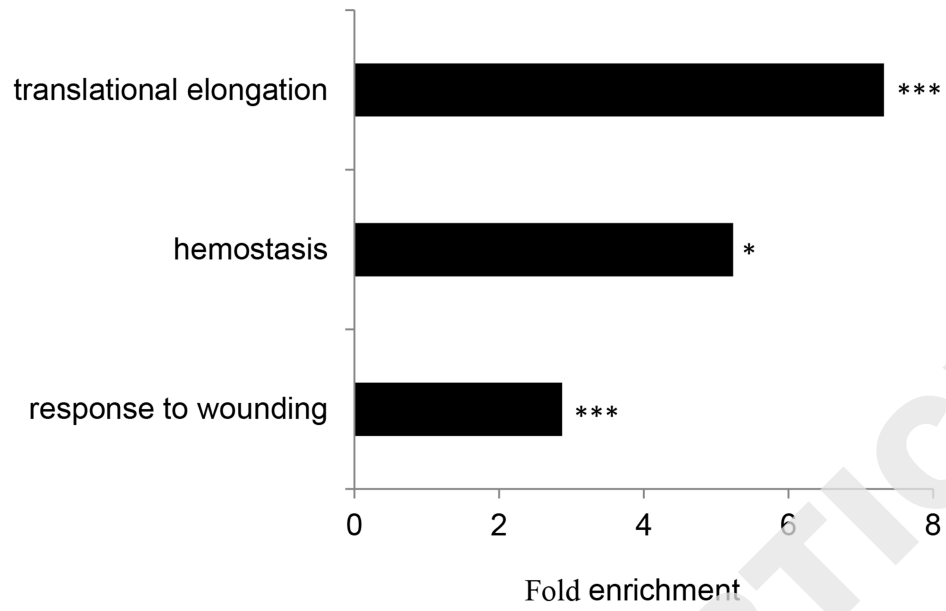
<sup>2</sup> Log ratio	Molecules	P adj.	Description
<b>Upregulated genes</b>			
1.824	CCNJL	2.0 x 10 <sup>-24</sup>	Cyclin J-Like
1.377	SHISA4	5.1 x 10 <sup>-6</sup>	Shisa Family Member 4
1.342	PLVAP	2.6 x 10 <sup>-6</sup>	Plasmalemma Vesicle Associated Protein
1.257	PDE5A	9.4 x 10 <sup>-8</sup>	Phosphodiesterase 5A
1.247	RIOK3	5.6 x 10 <sup>-8</sup>	RIO Kinase 3
1.247	TUBB1	7.8 x 10 <sup>-8</sup>	Tubulin, Beta 1 Class VI
1.211	ARHGEF12	1.3 x 10 <sup>-7</sup>	Rho Guanine Nucleotide Exchange Factor (GEF) 12
1.193	SH3BGL2	5.7 x 10 <sup>-7</sup>	SH3 Domain Binding Glutamate-Rich Protein Like 2

1.179	LINC00989	$7.3 \times 10^{-6}$	Long Intergenic Non-Protein Coding RNA 989
1.172	DAAM2	0.00012	Dishevelled Associated Activator Of Morphogenesis 2
1.169	CA2	$6.2 \times 10^{-6}$	Carbonic Anhydrase II
1.168	CTTN	$2.1 \times 10^{-6}$	Cortactin
1.165	RNF182	0.00027	Ring Finger Protein 182
1.163	ITGA2B	$2.7 \times 10^{-6}$	Integrin, Alpha 2b
1.151	EIF2AK1	$4.5 \times 10^{-7}$	Eukaryotic Translation Initiation Factor 2-Alpha Kinase 1
1.139	RAB27B	$2.1 \times 10^{-6}$	RAB27B, Member RAS Oncogene Family
1.136	PROS1	$4.8 \times 10^{-5}$	Protein S (Alpha)
1.123	CXCR2P1	$1.4 \times 10^{-5}$	Chemokine (C-X-C Motif) Receptor 2 Pseudogene 1
1.114	ABCG2	$7.8 \times 10^{-5}$	ATP-Binding Cassette, Sub-Family G (WHITE), Member 2
1.111	ITGB3	0.00014	Integrin, Beta 3 (Platelet Glycoprotein IIIa, Antigen CD61)
1.093	ARL4A	$3.0 \times 10^{-6}$	ADP-Ribosylation Factor-Like 4A
1.082	STON2	$7.8 \times 10^{-8}$	Stonin 2
1.081	CDKN1A	$2.1 \times 10^{-6}$	Cyclin-Dependent Kinase Inhibitor 1A
1.075	CTNNAL1	0.0009	Catenin (Cadherin-Associated Protein), Alpha-Like 1
1.073	CTDSPL	$6.7 \times 10^{-6}$	CTD (Carboxy-Terminal Domain, RNA Polymerase II, Polypeptide A) Small Phosphatase-Like
1.073	MYLK	$1.6 \times 10^{-5}$	Myosin Light Chain Kinase
1.062	ITGB5	$1.2 \times 10^{-5}$	Integrin, Beta 5
1.059	TUBB2A	0.0011	Tubulin, Beta 2A Class IIa
1.041	MFAP3L	$4.4 \times 10^{-6}$	Microfibrillar-Associated Protein 3-Like
1.031	MYOF	$4.2 \times 10^{-6}$	Myoferlin
1.030	GNAZ	$6.2 \times 10^{-6}$	Guanine Nucleotide Binding Protein (G Protein), Alpha Z Polypeptide
1.030	RNF10	$4.3 \times 10^{-6}$	Ring Finger Protein 10
1.029	MKRN1	0.0008	Makorin Ring Finger Protein 1
1.026	IFIT1B	0.0018	Interferon-Induced Protein With Tetratricopeptide Repeats 1B
1.025	LTBP1	$6.5 \times 10^{-5}$	Latent Transforming Growth Factor Beta Binding Protein 1
1.020	MMD	$4.2 \times 10^{-6}$	Monocyte To Macrophage Differentiation-Associated
1.019	GNG11	$4.8 \times 10^{-5}$	Guanine Nucleotide Binding Protein (G Protein), Gamma 11
1.011	ELOVL7	$2.9 \times 10^{-5}$	ELOVL Fatty Acid Elongase 7
1.011	HORMAD1	$8.4 \times 10^{-5}$	HORMA Domain Containing 1
<b>Downregulated genes</b>			
-1.014	SNORA80B	$1.3 \times 10^{-5}$	Small Nucleolar RNA, H/ACA Box 80B
-1.064	SCARNA21	$1.8 \times 10^{-6}$	Small Cajal Body-Specific RNA 21
-1.072	COL4A3	0.00017	Collagen, Type IV, Alpha 3
-1.091	SNORD10	$2.4 \times 10^{-8}$	Small Nucleolar RNA, C/D Box 10
-1.140	SNORA34	$6.7 \times 10^{-6}$	Small Nucleolar RNA, H/ACA Box 34
-1.167	SNORA71D	$4.0 \times 10^{-6}$	Small Nucleolar RNA, H/ACA Box 71D
-1.214	RPS18P9	$1.1 \times 10^{-6}$	Ribosomal Protein S18 Pseudogene 9
-1.397	SNORA49	$6.3 \times 10^{-9}$	Small Nucleolar RNA, H/ACA Box 49

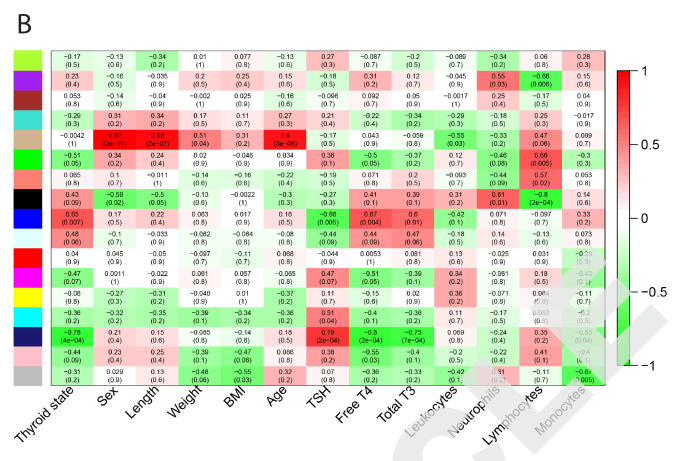
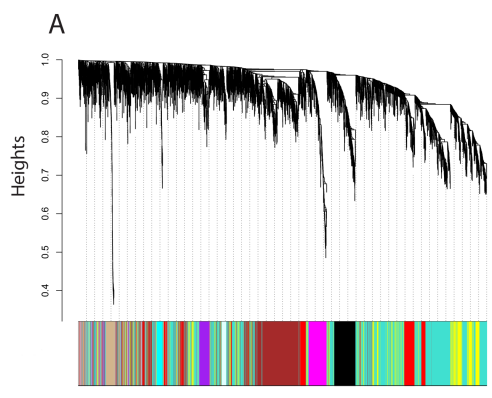


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