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Transcriptional Activity of Neurotrophins Genes and Their Receptors in the Peripheral Blood in Patients with Thyroid Diseases in Bukovinian Population of Ukraine

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Abstract

AIM: Thyroid hormone (TH) has an especially strong impact on central nervous system development and TH deficiency has been shown to result in severe mental retardation. It is crucial to identify compensatory mechanisms that can be involved in improving cognitive function and the quality of life of patients with hypothyroidism.

METHODS: We used the pathway-specific PCR array (Neurotrophins and Receptors RT^2 Profiler PCR Array, QIAGEN, Germany) to identify and validate neurotrophins genes and their receptors expression in patients with thyroid pathology and control group.

RESULTS: The analysis of gene expression of neurotrophins and their receptors showed that CRHBP, FRS2, FRS3, GFRA1, GFRA2, Glial maturation factor-beta (GMFB), nerve growth factor (NGF), NRG2, NRG4, NTF4, TRO, and VGF significantly decreased their expression in Group 3, which includes the patients with post-operative hypothyroidism. The patients with primary hypothyroidism stemming from AIT had significantly reduced expression of CRHBP, GFRA1, GFRA2, GMFB, NGF, PTGER2, and VGF, while the expression of NRG4 and TRO increased. In Group 3, which includes the patients with AIT and elevated serum anti-Tg and anti-TPO autoantibodies, the mRNA levels of GFRA2, NGF, NRG2, NTF4, NGF, and PTGER were reduced, and the expression of CRHBP, FRS2, FRS3 GFRA1, GMFB, NRG4, TRO, and VGF significantly increased.

CONCLUSION: These results indicate significant variability in the transcriptional activity of the genes of encoding for neurotrophins and their receptors in the peripheral blood in people with thyroid diseases.

Introduction

Hypothyroidism is a common condition with an incidence of 8% in the adult population [1]. Its clinical picture includes cognitive, attention, and mental disorders such as depression, suggesting hippocampal alterations [2]. Most symptoms usually recede following thyroid hormone (TH) replacement therapy, but some persist, especially in the cases of long-term hypothyroidism. TH has an especially strong impact on central nervous system (CNS) development, and TH deficiency has been shown to result in severe mental retardation [3]. While early identification and treatment using replacement therapy can prevent or ameliorate developmental defects, there is evidence that certain neurocognitive impairments may still persist [4]. Although acute effects of TH deficiency have been extensively studied, little is known about its long-term consequences on the cellular function or the capacity of the brain for complete recovery from postnatal hypothyroidism.

In previous studies, we demonstrated that autoimmune thyroiditis (AIT) and hypothyroidism can affect the transcription of mRNA for the genes involved in nerve impulse transmission and cell cycle in a gene-specific manner [5], [6], [7]. These changes in gene expression can also play a role in the development of neurological complications related to thyroid pathology, but the model of neurotrophin expression and its regulation under pathological conditions is not yet complete. Therefore, it is crucial to identify compensatory mechanisms that can be involved in improving cognitive function and the quality of life of patients with hypothyroidism.

While neurotrophins were initially described as modulators of cell growth and maintenance in the nervous and immune systems, their role in various pathophysiological conditions is now receiving considerable interest. Changes in neurotrophin expression within a tissue can indicate an ongoing pathophysiological process. Many genes for neurotrophins and their receptors are not only transcribed but also translated in blood cells (https://www.proteinatlas.org). Consequently, neurotrophins detected in the blood circulation system, and in their local expression, are assumed to reflect systemic neurotrophin levels. Transcriptional induction or

gene repression is an important indicator of the severity of pathological changes in tissues [8], [9], [10], [11]. This study aims to analyze the transcriptional activity of genes for neurotrophins and their receptors in peripheral blood cells in patients with thyroid gland diseases. Neurotrophin-specific biomarkers in the blood can be then used as a prognostic marker for the risk of developing neurological and psychological complications comorbid with thyroid pathology. We are using PCR arrays to determine the effect of TH and serum autoantibodies, such as anti-thyroglobulin (anti-TG) antibody and anti-thyroid peroxidase antibody (anti-TPO), on the transcription of genes encoding neurotrophins and their receptors in the patients with primary hypothyroidism stemming from AIT and post-operative hypothyroidism as well as the patients with AIT and elevated serum autoantibodies, such as anti-Tg and anti-TPO.

Methods

One hundred fifty-three patients with thyroid pathology were enrolled in the study. They were divided into 3 groups: Group 1 included 16 patients with post-operative hypothyroidism; group 2 included 65 patients with hypothyroidism resulting from AIT; and group 3 included 72 patients with AIT and elevated serum an anti-Tg and anti-TPO antibodies. Control group included 25 healthy individuals, which were recruited randomly, without matching for age or sex. Clinical characteristics of the subjects are shown in Table 1.

Hypothyroidism was diagnosed following the recommendations of the American Association of Clinical Endocrinologists 2012. The diagnosis of AIT was based on detected circulating antibodies to thyroid antigens (anti-TPO and anti-TG) and reduced echogenicity on thyroid sonogram in a patient with relevant clinical features [12].

Blood specimens were collected between 8 and 10 AM after an overnight fast. Free thyroxine (fT4) (normal range 6.0–13.0 pmol/L for males and 7.0–13.5 pmol/L for females), thyroid-stimulating hormone (normal range 0.3–4.0 mIU/mL), anti-TPO (normal range 0–30 IU/mL), and anti-TG (normal range 0–65 IU/mL) antibody levels were determined in every individual using STAT FAX303/Plus analyzer (Awareness Technology Inc, USA).

Patients under the age of 18 or those suffering from malignancy, inflammation associated rheumatic diseases or acute/chronic infection, diabetes mellitus, cardiovascular or cerebrovascular diseases, chronic hepatic or renal diseases, as well as pregnant women and those using any drugs that could interfere with thyroid function, were excluded from the study.

We used a pathway-specific PCR array (Neurotrophins and Receptors RT2 Profiler PCR Array, QIAGEN, Germany) to identify and verify cytokines and receptor pathways-associated gene expression in randomly selected 12 individuals from each group using real-time PCR due to the procedure described below.

Experimental procedures

RNA isolation

Total RNA was isolated from white blood cells using NucleoZOL (Macherey-Nagel, Germany) according to the manufacturer's instructions. NucleoZOL is designed for the isolation of total RNA (small and large RNA) in a single or separate fraction from a variety of sample materials, such as cells, tissue, and liquids of human or animal origin. White blood cells were lysed and homogenized in NucleoZOL reagent based on guanidinium thiocyanate and phenol.

cDNA synthesis

The RNA quality was determined and it was reverse transcribed. The concentration and quality of the isolated total RNA were determined on a NanoDrop spectrophotometer (Thermo Scientific™, USA). For the reverse transcription procedure with a cDNA conversion RT² First Strand Kit (QIAGEN, Germany, Cat. no. 330401), RNA samples with the following parameters were selected: Ratio A260/A280 within the range of 1.8-2.2.

The RT2 HT First Strand Kit procedure comprises two steps: Elimination of genomic DNA contamination and reverse transcription, which enable fast and easy handling of 96 RNA samples simultaneously. After genomic DNA elimination, the RNA sample undergoes reverse transcription with an RT master mix, as well as random hexamers and oligo-dT prime reverse transcription to capture more difficult-to-detect genes.

Table 1: Clinical characteristics of the subjects

Variable	Control group (n = 25)	Patients with post-operative hypothyroidism (Group 1) (n= 16)	Patients with hypothyroidism as a result of AIT (Group 2) (n = 65)	Patients with AIT with rising serum anti-Tg and anti-TPO autoantibodies (Group 3) (n = 72)
The age (years)	46.08 ± 14.58	47.30 ± 12.27	46.72 ± 15.49	45.02 ± 13.65
fT4 (pmol/L)	8.91 ± 0.97	3.44 ± 0.31	4.13 ± 0.52	8.51 ± 0.82
Thyroid-stimulating hormone (mIU/mL)	2.67 ± 0.52	8.61 ± 0.84	7.09 ± 0.50	2.38 ± 0.62
Anti-TPO (IU/mL)	34.04 ± 3.70	36.13 ± 2.78	380.62 ± 73.42	330.36 ± 50.23
Anti-TG (IU/mL)	15.32 ± 1.97	15.50 ± 1.90	32.97 ± 4.27	36.38 ± 7.70
Current dose of L-thyroxine (μg/day)	None	110.95 ± 5.25	88.46 ± 1.55	None

Data are expressed as mean ± standard deviation

PCR array

The cDNA was then used with RTI Profiler PCR Array (QIAGEN, Cat. no. PAHS-031Z) in combination with RTI SYBR® Green qPCR Mastermix (QIAGEN, Cat. no. 330504), following the complete RT2 Profiler PCR Array procedure (www.qiagen.com). Samples were assigned to control and study groups. CT values were normalized based on the automatic selection from the full panel of reference genes. Any Ct value >35 was considered to be a negative call. The RT2 Profiler, PCR Array data analysis software, calculates the fold change based on the widely used and agreed upon ΔΔCt method. The data analysis web portal calculates fold change/regulation using delta-delta CT method, in which delta CT is calculated between the gene of interest (GOI) and an average of reference genes (HKG), followed by delta-delta CT calculations (delta CT [Test Group]-delta CT [Control Group]). Fold change is then calculated using 2^ (-delta-delta CT) formula. This data analysis report was exported from the QIAGEN web portal at GeneGlobe. The software allows defining the best reference genes for normalization.

A list of neurotrophin and receptor pathway-focused genes selected for this research is given in Table 2.

Table 2: Neurotrophins and receptors pathway-focused genes

UniGene	RefSeq	Symbol	Description
Hs. 377783	NM_001118	ADCYAP1R1	Adenylate cyclase activating polypeptide 1 (pituitary) receptor type I
Hs. 115617	NM_001882	CRHBP	Corticotropin-releasing hormone-binding protein
Hs. 417628	NM_004382	CRHR1	Corticotropin-releasing hormone receptor 1
Hs. 729970	NM_001883	CRHR2	Corticotropin-releasing hormone receptor 2
Hs. 593446	NM_006654	FRS2	Fibroblast growth factor receptor substrate 2
Hs. 194208	NM_006653	FRS3	Fibroblast growth factor receptor substrate 3
Hs. 46894	NM_004960	FUS	Fused in sarcoma
Hs. 388347	NM_005264	GFRA1	GDNF family receptor alpha 1
Hs. 441202	NM_001495	GFRA2	GDNF family receptor alpha 2
Hs. 151413	NM_004124	GMFB	Glia maturation factor, beta
Hs. 5210	NM_004877	GMFG	Glia maturation factor, gamma
Hs. 5258	NM_006986	MAGED1	Melanoma antigen family D, 1
Hs. 2561	NM_002506	NGF	Nerve growth factor (beta polypeptide)
Hs. 7303	NM_022002	NR1I2	Nuclear receptor subfamily 1, group I, member 2
Hs. 408515	NM_013982	NRG2	Neuregulin 2
Hs. 732438	NM_138573	NRG4	Neuregulin 4
Hs. 266902	NM_006179	NTF4	Neurotrophin 4
Hs. 2090	NM_000956	PTGER2	Prostaglandin E receptor 2 (subtype EP2), 53kDa
Hs. 633653	NM_016157	TRO	Trophinin
Hs. 587325	NM_003378	VGF	VGF nerve growth factor inducible

Statistical analysis of PCR array data

The RT2 Profiler PCR Array Data Analysis software does not perform any statistical analysis beyond the calculation of p-values using a Student's t-test (two-tail distribution and equal variances between the two samples) based on the triplicate 2^(–ΔCT) values for each gene in the experimental group compared to the control group. The Microarray Quality Control published results indicate that a ranked list of genes based on fold-change and associated p-value calculation was sufficient to demonstrate reproducible results across multiple microarrays and PCR Arrays, including the RT2 Profiler PCR arrays.

Ethical approval

The ethical principles contained in the Declaration of Human Rights adopted in Helsinki in 1975, and revised in 2008, were fully respected in our study. The subjects enrolled voluntarily participated in this study and completed and signed written informed consent. The protocol of study was approved by the local ethics committees of I. Horbachevsky Ternopil National Medical University and Chernivtsi Regional Endocrinology Center.

Results

Using the Pathway-Focused PCR Array Profiling (Neurotrophins and Receptors RT2 Profiler PCR Array), we examined the neurotrophins and receptors pathway-focused genes expression of patients with primary hypothyroidism as a result of AIT and post-operative hypothyroidism and patients with AIT with rising serum autoantibodies, such as anti-Tg and anti-TPO.

The results from RT2 Profiler neurotrophins and receptors pathway-focused genes expression analysis indicated that in Group 1, which includes patients with post-operative hypothyroidism, the expression of a lot of genes was decreased compared with other groups of patients (Table 3). Reductions in CRHBP (5.03-fold), FRS2 (3.3-fold), FRS3 (2.7-fold), GFRA1 (5.5-fold), and GFRA2 (4.3-fold) mRNAs were found in Group 1 (Figure 1). The expression of glial maturation factor-beta (GMFB) (4.6-fold), nerve growth factor (NGF) (7.8-fold), and NRG2 (4.6-fold) were markedly decreased too in Group 3 (Table 3). As it is shown in Table 3, reductions in NRG4 (3.8-fold), NTF4 (3.6-fold), TRO (6.9- fold), and VGF (5.03-fold) were also found in Group 1. In contrast, the expression of PTGER2 (2.9-fold) was increased (Figure 1).

Table 3: Differential expression of mRNA neurotrophins and receptors pathway-focused genes in patients with different thyroid pathology

Symbol	Up-down regulation (comparing to control group)		
	Group 1	Group 2	Group 3
	Fold regulation	Fold regulation	Fold regulation
ADCYAP1R1	– 1.2988 (p = 0.19)	1.0694 (p = 0.74)	1.03 (p = 0.88)
CRHBP	– 5.0298 (p = 0.000153)	– 4.5416 (p = 0.000089)	7.0422 (p = 0.005)
CRHR1	– 1.0525 (p = 0.204006)	– 1.052 (p = 0.435348)	– 1.0772 (p = 0.061173)
CRHR2	– 1.05 (p = 0.204)	– 1.05 (p = 0.435)	– 1.08 (p = 0.06)
FRS2	– 3.29 (p = 0.01)	– 1.25 (p = 0.288)	12.17 (p = 0.025)
FRS3	– 2.733 (p = 0.008)	– 1.248 (p = 0.099)	7.742 (p = 0.04)
FUS	1.0789 (p = 0.648)	1.1702 (p = 0.324)	– 1.4647 (p = 0.198)
GFRA1	– 5.49 (p = 0.0002)	– 5.92 (p = 0.0015)	3.45 (p = 0.012)
GFRA2	– 4.32 (p = 0.00038)	– 3.94 (p = 0.0016)	– 3.66 (p = 0.0017)
GMFB	– 4.63 (p = 0.0145)	– 5.96 (p = 0.0036)	13.42 (p = 0.0126)
GMFG	– 1.14 (p = 0.343075)	– 1.59 (p = 0.298804)	– 1.32 (p = 0.096805)
MAGED1	– 1.05 (p = 0.204006)	– 1.05 (p = 0.435348)	– 1.077 (p = 0.061173)
NGF	– 7.84 (p = 0.0007)	– 3.83 (p = 0.001)	– 4.076 (p = 0.0009)
NRG2	– 4.63 (p = 0.001)	– 1.127 (p = 0.365)	– 4.65 (p = 0.002)
NRG4	– 3.847 (p = 0.007)	8.92 (p = 0.004)	14.41 (p = 0.003)
NTF4	– 3.58 (p = 0.0036)	– 1.089 (p = 0.875)	– 3.78 (p = 0.0038)
PTGER2	2.94 (p = 0.0059)	– 5.72 (p = 0.001)	– 6.15 (p = 0.0005)
TRO	– 6.95 (p = 0.002)	6.44 (p = 0.0008)	7.37 (p = 0.0001)
VGF	– 5.03 (p = 0.0004)	– 4.73 (p = 0.0003)	6.03 (p = 0.002)

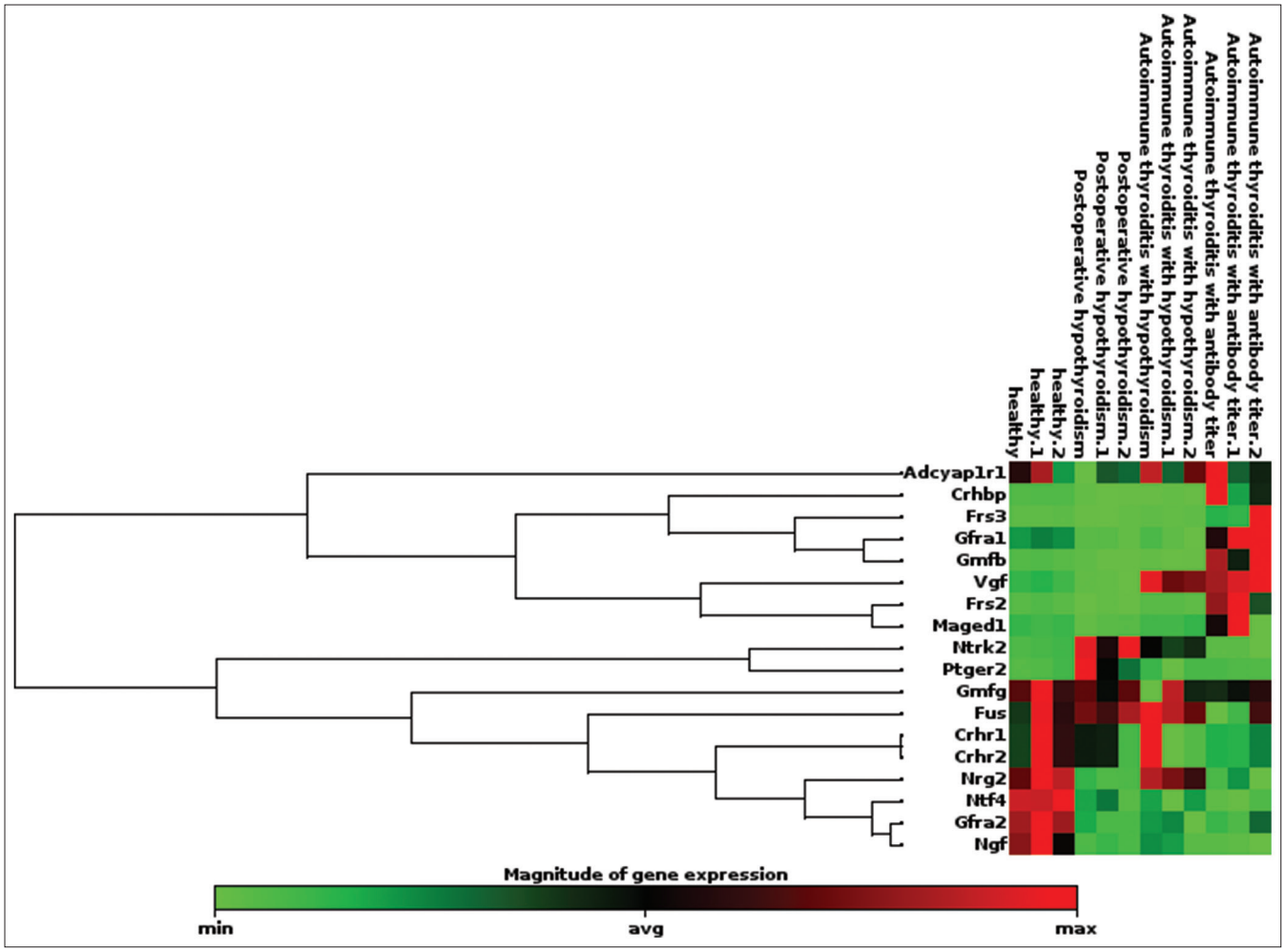

Figure 1: Clustergram expression of mRNA Neurotrophins & receptors pathway-focused genes in patients with different thyroid pathology

In patients with hypothyroidism as a result of AIT (Group 2), the expression of neurotrophins and receptors pathway-focused genes changed as follows: The decrease in the expressions of CRHBP (4.5-fold), GFRA1 (5.9-fold), GFRA2 (3.9-fold), and GMFB (5.9-fold) were observed. As it is shown in Table 3, reductions in NGF (3.8-fold), PTGER2 (5.7-fold), and VGF (4.7-fold) mRNAs were also found in Group 2, whereas the expressions of TRO (6.4-fold) and NRG4 (8.9-fold) were increased (Figure 1).

We noted that in Group 3 which includes patients with AIT with rising serum anti-Tg and anti-TPO autoantibodies that mRNA level of CRHBP (7.04-fold), FRS2 (12.2-fold), and FRS3 (7.7-fold) were significantly increased (Figure 1). Reductions in GFRA2 (3.7-fold), NGF (4.1-fold), NRG2 (4.7-fold), NTF4 (3.8-fold), and NGF (4.1-fold) PTGER (6.1-fold) mRNAs were found in Group 3. The expression of GFRA1 (3.5-fold), GMFB (13.4-fold), and NRG4 (14.4-fold) were markedly increased in Group 3 (Figure 1). What is more, the expression of TRO (7.4-fold) and VGF (6.03-fold) was increased too (Figure 1).

Besides, we found that ADCYAP1R1, CRHR1, CRHR2, FUS, GMFG, and MAGED1 did not change their expression in all groups of patients.

The p-values are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control group and patients groups.

Discussion

Neurotrophins are a family of neurotrophic factors essential for the development of the vertebrate nervous system. They regulate neuron differentiation, survival, or death during embryonic and postnatal development and are involved in neuronal maintenance later in life [13]. The first such substance to be identified, and the prototype of the class was NGF [14]. The neurotrophins are expressed in a broad array of tissues, consistent with the view that they mainly function as target-derived survival factors [15].

Although the developmental effects of TH have been well established, its impacts on the adult brain are relatively poorly understood [16]. While the adult mammalian brain does not exhibit the severe morphological defects associated with developmental hypothyroidism, TH deficiency in adulthood has

been linked to cognitive dysfunction and depressed mood [17]. For example, adult-onset hypothyroidism in humans is linked to impaired learning, verbal fluency, and spatial tasks [18], as well as an increased risk of depression [19]. This suggests that thyroid dysfunction in adulthood may result in morphological changes to the brain regions associated with learning, memory, and mood, such as the hippocampus.

In many body tissues, neurotrophins are produced by a variety of non-neuronal cell types such as immune cells, adipocytes, endothelia, epithelia, fibroblasts, keratinocytes, and endocrine cells [20]. Variations in neurotrophin concentrations found in the systemic blood circulation indicate their both vascular and peripheral production. Neurotrophins can cross through blood-brain barrier [21], [22], although the peripheral expression of neurotrophic factors in different tissues (the thymus, heart, liver, pancreas, spleen, kidney, and adrenal glands) has been also reported [23], [24]. It has been suggested that altered RNA and protein expression of the neurotrophic factors in the peripheral tissues can indicate brain disorders [25].

Corticotropin-releasing hormone (CRH) is a key regulator of the stress response [26]. This peptide controls the hypothalamic-pituitary-adrenal axis as well as a variety of behavioral and autonomic stress responses. The CRH system in vertebrates includes two receptors (CRH-R1 and CRH-R2), which show dissimilar expression patterns in the brain and periphery [27]. We found that CRH-R1 and CRH-R2 expressions did not significantly change in all groups of patients.

The CRH system also involves an evolutionarily conserved corticotropin-releasing factor-binding protein (CRHBP), a high-affinity binding protein that modulates CRH-mediated activation of CRH receptors in the brain and periphery and the primary mediator of the mammalian neuroendocrine and behavioral response to stress [28], encoded by CRHBP gene. In humans, CRHBP is widely distributed throughout the body and is found in several brain regions, including the cerebral cortex, the hippocampus, amygdala, lateral septal nucleus, and a variety of midbrain structures [29]. Early studies indicated that approximately 40–60% of CRH in the human brain is bound by CRHBP, suggesting its role in limiting the bioavailability of CRH and reducing CRH receptor activation [30]. One study found that CRHBP plasma levels were elevated in inflammatory conditions such as rheumatoid arthritis and septicemia, indicating that CRHBP may be positively regulated by inflammatory stressors [26]. In this study, we found a significant decrease in the expression of CRHBP in Groups 1 and 2. On the other hand, in Group 3, the expression of CRHBP was increased. This suggests suppression of CRHBP expression in the cases of TH deficiency resulting from AIT and post-operative hypothyroidism.

Fibroblast growth factor (FGF) receptor substrates 2 and 3 (FRS2 and FRS3) are two related adapter proteins, sharing 49% sequence identity and activated by the FGF and NTRK1 receptors [31]. Studies suggest that FRS2 and FRS3 transducers are involved in the thyroid tumorigenesis induced by TRK oncogenes and thus might represent targets for treatment approaches aimed at blocking oncoprotein signaling [32]. Certain factors such as neurotrophins and FGFs can play both neurogenic and synaptic roles [33], [34], [35]. Neurotrophins and FGFs promote postnatal dentate neurogenesis by signaling through their specific receptor subtypes, FGF Receptor 1 (FGFR1), and Neurotrophic Tyrosine Kinase Receptor type 2 (NTRK2 or TrkB) [36]. Specific FGF receptor isoforms (FGFR1b and FGFR2b) are involved in synaptogenesis in CA3 pyramidal neurons, while TrkB was implicated in DGC maturation [34], [35]. In this study, FRS2 and FRS3 expression significantly decreased in the group of patients with post-operative hypothyroidism, while it significantly increased Group 3, which includes patients with AIT and elevated serum anti-Tg and anti-TPO autoantibodies.

A new family of neurotrophic factors composed of four members, namely, GDNF, NTN, ART, and PSP which other preferentially to GFRa-1, GFRa-2, GFRa-3, and GFRa-4, respectively, has been recently described [37]. Within the CNS, GFRa-1 and GFRa-2 show a widespread expression [38]. A dramatic increase in GFRa-2 mRNA that was triggered after short-term treatment with THs suggests a specific action through high affinity receptors for this gene [37]. TH receptors were detected in glial cells [39] and T3 affects the development of both astrocytes [40] and oligodendrocytes [41], indicating that T3 may control the expression of GFRa2 in these two cell types.

The results of our study indicated that the expression of GFRA2 was significantly decreased in all groups of patients. At the same time, the patients with primary hypothyroidism resulting from AIT and post-operative hypothyroidism had significantly lower expression of GFRA1, while in the group of patients with AIT and rising serum anti-Tg and anti-TPO autoantibodies, the levels of GFRA1 mRNA were significantly increased.

GMFB is a highly conserved brain-enriched protein implicated in immunoregulation, neuroplasticity, and apoptosis, processes central to neural injury and repair following cerebral ischemia. Although GMFB is expressed in multiple organs, the expression is enriched in brain tissue and relatively stable in adults [42], [43]. Plasma GMFB levels can serve as a convenient non-invasive addition to neuroimaging for stroke diagnosis and prognosis [44]. The expression of GMFB is also altered in several neurodegenerative diseases [45], suggesting that GMFB as a broadly applicable disease biomarker. Peak GMFB expression correlates with learning and memory formation in rats [46]. Further, the neurite localization of this protein is also consistent with its role in neural plasticity. In this study, we found

that GMFB was downregulated in Groups 1 and 2. Patients with AIT and rising serum autoantibodies had significantly higher expression of GMFB.

The biological effect of the neurotrophins is mediated through the high-affinity tropomyosin-related family of tyrosine receptor kinases (TrkA, TrkB, and TrkC) and the p75 neurotrophin receptor which can bind all neurotrophins with low affinity [21]. These receptors can either enhance or inhibit each other's actions to mediate neurotrophic effect. NGF, neurotrophin (NT-3), and NT-4 are expressed by neurons, microglial cells, and astrocytes, as well as activated lymphocytes [47]. NT-4 levels were shown to correlate with psychosocial functioning in patients with bipolar disorder in remission [48]. A recent meta-analysis conducted by Tseng *et al*. found increased NT-3 and NT-4 levels in patients with bipolar disorder in a depressed state when compared to healthy controls. This difference was significantly associated with the duration of illness [48].

Different inflammatory and autoimmune diseases lead to altered expression of NGF. Increased anti-NGF antibody levels have been detected in patients with rheumatoid arthritis, systemic lupus erythematosus, and thyroiditis and are thought to contribute to the immune dysfunction and nerve damage observed in these diseases [49]. NGF is expressed not only in the neuronal but also non-neuronal cells (epithelial, endothelial and skeletal muscle cells, fibroblasts, adipocytes, and bone marrow-derived cells); it can modify the local immune responses promoting T-helper 2 dominance with the release of various cytokines, chemokines, and prostaglandin derivatives [50]. NGF is involved in both neuronal cell function and inflammatory-immune cell activity, contributing to the development and maintenance of chronic inflammation [51]. NGF secretion increases in hypothyroidism [52]. In our study, we found reduced levels of NGF mRNA in all groups of patients. This is in contrast to the expression of NTF4, which was reduced only in Groups 1 and 3.

The neuregulins (NRGs) are cell-cell signaling proteins that are ligands for tyrosine kinase receptors of the ErbB family. The neuregulin gene family has four members: NRG1, NRG2, NRG3, and NRG4 [53]. NRGs and their receptors are widely expressed in the postnatal nervous system [54]; NRG expression in the brain is upregulated by its activity; and NRGs can inhibit long-term potentiation (LTP), a mechanism involves in learning [55]. In this study, we determined that patients with post-operative hypothyroidism had a significantly increased expression of NRG2 and NRG4. The NRG2 mRNA levels decreased, while NRG4 mRNA levels significantly increased in the patients with elevated serum autoantibodies anti-Tg and anti-TPO.

EP2 receptor plays an important role in hippocampal LTP and spatial learning. PGE2 induces a synaptic response through its action on a pre-synaptic EP2 receptor [56]. This means that PGE2-EP2 signaling is important for hippocampal long-term synaptic plasticity and cognitive function. In this study, PTGER2 mRNA increased in Group 1 which included patients with post-operative hypothyroidism, while PTGER2 expression decreased in the group of AIT patients with elevated serum autoantibodies, such as anti-Tg and anti-TPO and in the patients with hypothyroidism as a result of AIT.

Trophinin (TRO) and bystin are highly expressed in the SVZ and RMS of the adult rat brain [57]. Since TRO is expressed on plasma membranes and in the cytoplasm of type C cells in the SVZ and in migrating neuroblasts in the RMS, it is possible that it interacts with the extracellular matrix promoting chain migration of neuroblasts along the RMS toward the olfactory bulb. We observed a decrease in expression of TRO in patients with post-operative hypothyroidism. On the contrary, TRO expression considerably increased in Groups 2 and 3.

VGF nerve growth factor inducible (VGF) was first identified as a neuropeptide, the expression of which is induced by NGF [58]. VGF expression is also induced by several other growth factors, such as brain-derived neurotrophic factor (BDNF) and NT-3 [59]. VGF is synthesized in neuronal and neuroendocrine cells as well as in the CNS, especially in the cerebral cortex, hippocampus, and hypothalamus [60], [61]. Studies show that VGF-derived peptides are involved in a number of functions in the CNS and peripheral tissues. For instance, in patients with the major depressive disorder who were drug-free for at least 2 weeks, VGF mRNA levels in leukocytes were significantly reduced, by approximately 50%, compared to healthy subjects [62]. In another study, VGF levels in serum and prefrontal cortex decreased in patients with the major depressive disorder [63]. In this study, patients with hypothyroidism as a result of AIT and post-operative hypothyroidism VGF expression was reduced, while it was significantly increased in the group of AIT patients with elevated serum autoantibodies, such as anti-Tg and anti-TPO.

Conclusion

In summary, the analysis of gene expression of neurotrophins and their receptors showed that CRHBP, FRS2, FRS3, GFRA1, GFRA2, GMFB, NGF, NRG2, NRG4, NTF4, TRO, and VGF significantly decreased their expression in Group 3, which includes the patients with post-operative hypothyroidism. The patients with primary hypothyroidism stemming from AIT had significantly reduced expression of CRHBP, GFRA1, GFRA2, GMFB, NGF, PTGER2, and VGF, while the expression of NRG4 and TRO increased. In Group 3, which includes the patients with AIT and elevated serum anti-Tg and anti-TPO autoantibodies, the

mRNA levels of GFRA2, NGF, NRG2, NTF4, NGF, and PTGER were reduced, and the expression of CRHBP, FRS2, FRS3 GFRA1, GMFB, NRG4, TRO, and VGF significantly increased. In addition, the expression of ADCYAP1R1, CRHR1, CRHR2, FUS, GMFG, and MAGED1 did show a significant change in all groups of patients. These results indicate significant variability in the transcriptional activity of the genes of encoding for neurotrophins and their receptors in the peripheral blood in people with thyroid diseases.

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