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Higher CSF/serum free-T4 ratio is associated with improvement of quality of life during treatment with L-thyroxine

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Abstract

Up to 20% of individuals with primary hypothyroidism treated with L-thyroxine still suffer from severe symptoms. These are supposedly brain derived and involve both cognitive and emotional domains. Previously, no consistent relationship has been found between thyroid hormones (TH) or TSH levels in blood and quality of life (QoL). Recently, we reported an association between cerebrospinal fluid (CSF)/serum free-thyroxine (f-T4) ratio and QoL, in juvenile hypothyroid patients. Here, we investigated if CSF/serum f-T4 ratio and QoL estimates correlate also during L-thyroxine treatment. Moreover, the CSF biomarker neurogranin (Ng) was used as a biomarker for synaptic function and integrity in clinical research. Ng is partially controlled by TH and therefore we investigated the relationship between QoL parameters and Ng levels. Patients diagnosed with primary

hypothyroidism were investigated using vital parameters, serum and CSF analyses of TH, TSH, Ng and QoL questionnaires. Similar procedures were performed after 6 months of treatment. The most marked associations with QoL were found for CSF/serum f-T4 ratio, which was strongly related to several QoL parameters such as the mental subscore of SF-36 ($r = 0.83$, $p < .0005$). Ng, which did not differ from that in our healthy controls, was lower in some patients during treatment and higher in others. However, the change in Ng during treatment was significantly correlated with QoL parameters including the mental subscore of SF-36 ($r = -0.86$, $p < .0001$). In addition, the CSF/serum f-T4 ratio correlated with the change in Ng ($r = -0.75$, $p = .001$). Our results suggest that the ratio between CSF and serum f-T4 is an important biomarker for QoL during treatment of patients with primary hypothyroidism, so far in research, but in the future maybe also in clinical settings. Moreover, this ratio also correlates with the changes in Ng levels during L-thyroxine treatment, further supporting the impact of the TH balance between serum and CSF on QoL.

KEYWORDS
cerebrospinal fluid biomarkers, cognition, neurogranin, primary hypothyroidism, quality of life

1 | INTRODUCTION

Primary hypothyroidism (PH) is one of the most common autoimmune diseases[1] affecting up to 4% of the adult population in Sweden.[2] There is a five-fold higher prevalence in females, and PH is widely included in screening protocols for symptoms such as fatigue and depressive mood.[3–7] PH is associated with a broad array of clinical symptoms as thyroid hormones affect virtually all the cells in our body. The symptoms include muscle pain, decreased sweating, peripheral edema and CNS symptoms such as fatigue, depressive state, and cognitive deficits.[8,9] Most of these symptoms will recede following levothyroxine (L-thyroxine) treatment. However, despite adequate concentrations of free iodothyronine (f-T3) and free thyroxine (f-T4) in peripheral blood,[10,11] approximately 10%–20% of patients will have some residual symptoms and several studies show that quality of life (QoL) will not return to the levels seen in healthy controls.[12–15]

Thyroid-stimulating hormone (TSH) is increased in untreated PH due to lack of sufficient feedback inhibition from T4 and T3 and the measurement of TSH in peripheral blood is used as a biomarker to assess the disease status. However the reference interval for TSH in healthy individuals, which is used to adjust the dose of L-thyroxine in patients with PH, is to a large extent based on clinical practice rather than on randomized controlled trials.[16] Furthermore, it has been reported that the level of TSH is not related to the QoL as experienced by patients receiving L-thyroxine treatment.[4,17] Substitution with L-thyroxine and the subsequent lowering of TSH decreases the activity of the type II iodothyronine deiodinase-enzyme (DIO2) by downregulation of the synthesis.[18] This could possibly explain why serum(s) T3/T4 ratio, both in terms of total and free fractions, of the hormones are lower during treatment as compared with healthy controls, since DIO2 activity is downregulated by the excess T4 concentration.[19,20] These lower T3/T4 ratios have been inversely associated with physical performance[6] and mood.[21] We recently reported that low cerebrospinal fluid (CSF)/serum f-T4 ratio is correlated with QoL parameters in patients with untreated PH, but not in healthy controls, indicating that also the ratio of TH over the blood–brain-barrier (BBB) is of importance.[22]

Neurogranin (Ng) has been proposed as a potential biomarker of synaptic function and integrity. Its transcription is regulated by thyroid hormones,[23,24] and knockout of the Ng-encoding *NGRN* gene in mice results in inability to produce Ng which entails impaired spatial memory, which can at least in part be reversed by the administration of L-thyroxine.[25] However, there is, as yet, no data for patients with PH showing that Ng could potentially be useful as a biomarker of central nervous functions and synaptic dysfunctions.[26]

In summary, many patients suffer from residual symptoms during L-thyroxine treatment despite normalized serum TH levels. It is therefore important to further study the mechanisms underlying the residual symptoms in PH. The main aim of the present study was to evaluate whether the CSF/serum f-T4 ratio is associated with QoL during treatment with L-thyroxine. A second aim was to investigate whether Ng levels are associated with the CSF/serum f-T4 ratio and QoL parameters. The results showed an association between CSF/serum fT4 ratio and QoL, and both these variables were in turn related to changes in Ng.

2 | MATERIALS AND METHODS

2.1 | Study population

Patients with PH ($n = 15$, 11 women), mean (M) age: 52 ± 12.2 were recruited at the Unit of Endocrinology at the Department of Medicine, Halland Central Hospital, Sweden (Table 1). The patients had been

TABLE 1 Clinical characteristics in hypothyroid patients before and during treatment, as well as in healthy controls.

	Before treatment M ± SD	During treatment M ± SD	Paired *t*-test	Healthy controls M ± SD	Unpaired *t*-test Before treatment vs. healthy controls
Gender, female/male	11/4	11/4		26/24	
Age	52.0 ± 12.2	52.6 ± 12.3	n.s.	36.4 ± 11.1	***
BSA (m^2)	2.0 ± 0.3	1.9 (1.7–2.2)	n.s.	1.8 ± 0.4	n.s.
Time between visit one and two (days)		231.5 ± 141.3			
s-TSH (mIU/L)	8.5 ± 9.7	0.8 ± 0.9	***	2.1 ± 1.1	***
s f-T4 (pmol/L)	11.3 ± 2.6	17.1 ± 3.6	***	14.1 ± 1.9	***
CSF-f-T4 (pmol/L)	8.5 ± 2.2	13.1 ± 2.5	***	9.6 ± 1.2	n.s.
s f-T3 (pmol/L)	5.4 ± 1.0	6.3 ± 1.3	*	6.2 ± 1.1	*
CSF f-T3 (pmol/L)	2.5 ± 0.9	2.0 ± 0.7	n.s.	2.3 ± 0.6	n.s.
sTPO-Ab >15 IU/mL	10 (67%)	10 (67%)		6 (12%)	
sTPO-Ab >25 IU/mL	8 (53%)	9 (60%)		6 (12%)	
Zulewski[27] score ≥5	15/15	4/15	***	0/50	***

Note: One hypothyroid patient did have an s-TSH of 43.2 before treatment.
Abbreviations: BSA, body surface area; f-T3, free iodothyronine; f-T4, free thyroxine; s-TSH, serum thyroid-stimulating hormone; TPO-Ab, thyroid peroxidase antibodies.

invited by their primary physician to participate in the study. Out of screened patients there were only two who did not want to participate, for which we do not have any characteristics. All patients displayed at least two pathologically increased TSH (>4.0 mIE/L; reference interval: 0.40–4.0 mIE/L) prior to admission. In addition, all patients had a f-T4 level in the lower-half of the reference interval (which was 11–22 pmol/L) with a mean of 11.3 ± 2.6 pmol/L. Furthermore, they showed more than five points according to the Zulewski scale[27] to ensure that they were clinically hypothyroid. The second visit occurred 182 (119–623) days after the first visit. The basal patient parameters are presented in Table 1.

All 25 hypothyroid patients described in our previous study[22] were invited to a second visit. Ten patients did not attend the second visit as three had moved out of our region, four had experienced post-puncture headache or discomfort after the initial lumbar puncture, one was pregnant, one was severely ill, and one was unreachable. The patients had a regular contact with the principal investigator of the study to make sure of compliance. Regular blood sampling was also done during the study period to make sure that the TSH levels did decrease.

Exclusion criteria were treatment with antithyroid substances or thyroid hormones, and recent use of iodine contrast or pregnancy. In all 15 patients, recent onset of fatigue, hypersomnia or lethargy was the main reason for consulting their primary physician. Apart from PH, one subject was previously diagnosed with type-1 diabetes, four patients were treated with estrogen hormone replacement (oral estrogen or patch), and one subject received glucocorticoid treatment (i.e., budesonid inhalation). None of the 15 patients fulfilled any criteria of a depressive diagnosis based on medical record review and clinical assessment at the visit. All patients were of Caucasian origin.

Since there are no normal reference values for TH in CSF, we present data for all our healthy controls. In the study they are referred as a reference group. They were studied at one visit. The reference group ($n = 50$, 26 women) were recruited by posters in the staff rooms at the medical clinic, ambulance, local senior citizen association, and the University of Halmstad. All controls were Caucasians, had TH and TSH levels within the reference intervals, and a Zulewski score[27] of 0–4 points. One person in the reference group had to be excluded due to a very high neurogranin value.

2.2 | Ethical considerations

The study was approved by the Regional Ethical Committee in Lund, Sweden (diary number 253/2008, 585/2009 and 491/2010). Oral and written informed consent was obtained from all participants.

2.3 | Bodyweight, height and area

Bodyweight was measured in the morning to the nearest 0.1 kg and body height was determined barefoot to the nearest 0.01 m. Body surface area (BSA) was calculated according to the Du Bois formula.

2.4 | Sampling of blood and CSF

All sampling of blood and CSF was performed after a minimum of 8 h fasting. Venous blood samples were obtained by venipuncture, centrifuged at 2400 *g* for 10 min between 1 and 3 h after sampling and serum were aliquoted in polypropylene tubes and stored at −20°C pending biochemical analyses of TSH, f-T3, f-T4, and thyroid peroxidase antibodies (TPO-Ab).

TABLE 2 Mean ± SD for thyroid hormones, neurogranin and quality of life (QoL).

Descriptive data results	Before treatment $n = 15$ M ± SD	During treatment $n = 15$ M ± SD	*p*-value for paired *t*-test	Healthy controls $n = 50$ M ± SD	*p*-value unpaired *t*-test before treatm–healthy controls
MADRS	11.3 ± 6.9	7.9 ± 6.3	.0047	(not available)	
General health Likert scale	66.5 ± 15.3	72.3 ± 17.9	0.0459	87.7 ± 10.0	.00008
Mean physical subscore SF-36	80.1 ± 24.2	90.2 ± 23.6	n.s.	109.2 ± 10.0	.0004
Mean mental subscore SF-36	68.9 ± 22.9	82.5 ± 22.6	.0126	98.9 ± 10.8	.0002
CSF/s f-T4	0.75 ± 0.09	0.78 ± 0.12	n.s.	0.69 ± 0.11	.0280
CSF/s f-T3	0.46 ± 0.18	0.33 ± 0.12	.0547	0.38 ± 0.13	n.s.
s f-T3/s f-T4	0.51 ± 0.14	0.37 ± 0.05	.0017	0.44 ± 0.07	n.s.
CSF f-T3/CSF f-T4	0.33 ± 0.20	0.16 ± 0.06	.0140	0.24 ± 0.07	n.s.
CSF neurogranin (pg/mL)	236.5 ± 228.5	218.8 ± 162.1	n.s.	155.2 ± 165.1	n.s.

Note: QoL parameters are Likert scale of general health (GHLS), MADRS and subscores of SF-36 (mental subscore [MCSc] and physical subscore [PCSc]).
Abbreviations: MADS, Montgomery-Åsberg Depression Rating Scale.

Lumbar puncture was performed at 08:00–08:30 a.m. according to a standardized procedure at the L4-5 interspace in the seated position, with a disposable needle (Becton-Dickinson, Quincke: 0.70 × 75 mm, 22 GA). A total of 12 mL CSF was collected in polypropylene tubes and divided in six 2 mL aliquots. The CSF samples were immediately transported to the local laboratory and centrifuged at 2000 *g* at +4 °C for 10 min and the supernatants were then stored at −70°C, pending biochemical analyses without being thawed and refrozen.

2.5 | Biochemical procedures

All serum and CSF samples were analyzed in the same assay run to minimize the analytical interassay variation. TSH, f-T3 and f-T4 were analyzed by dissociation-enhanced lanthanide fluoroimmunoassays[28] (Auto DELFIA). The intra-assay coefficient of variation (CV%) for the TSH assay was <4.4% for all levels down to 0.1 mIE/l, and the intra-assay CVs for the f-T4 and f-T3 assays were <6.1% for all levels down to five and two pmol/L, respectively. We chose the free component of TH and not the total as in the clinical setting it is most relevant to use serum f-T4 and not serum T4. TPO-Ab were analyzed by chemiluminescent microparticle immunoassay (CMIA) (Architect i2000, Abbott). The intra-assay CV for the TPO antibody assay was <10% for all levels down to 5 IU/mL; >15 IU/mL was considered as a positive result.

CSF neurogranin (Ng) was measured using an inhouse enzyme-linked immunosorbent assay (ELISA), based on the Ng2 and Ng22 monoclonal antibodies, as previously described.[29]

2.6 | Quality of life

After lumbar puncture and venous blood sampling had been performed, all patients completed the self-assessment of general health using a Likert scale (GHLS)[30] as well as the Montgomery-Åsberg Depression Rating Scale (MADRS) questionnaire to evaluate symptoms of depression.[31] SF-36[32] was used to calculate the sum score for physical health (PCSc) and for mental health (MCSc) as previously described.[33]

All QoL assessments were performed both before and after in mean 6 months of L-thyroxine treatment and we could therefore calculate the change between them (during-before).

2.7 | Statistical methods

We used the R 4.0.5 software for statistical analyses. The biomarker and test results are presented as the mean ± SD. We used the Shapiro test and QQ-plots to confirm that the data was normally distributed. The delta values, with both positive and negative values, were transformed with the power of 10 transformation. Spearman's rank correlation coefficient was sought for correlation analysis. A paired *t*-test was used to calculate within-group effects (comparing before and during treatment). A two-tailed *p*-value <0.05 was considered statistically significant. *p*-values are marked in tables with * according to the international standard for significance level. Since we have looked at several parameters of TH we adjusted the *p*-values using the Holm method with seven TH values.

3 | RESULTS

3.1 | Quality of life

The patients improved in most QoL parameters during treatment (Table 2 and Figure 1). MADRS score was reduced, GHLS and MCSc were increased, and PCSc tended

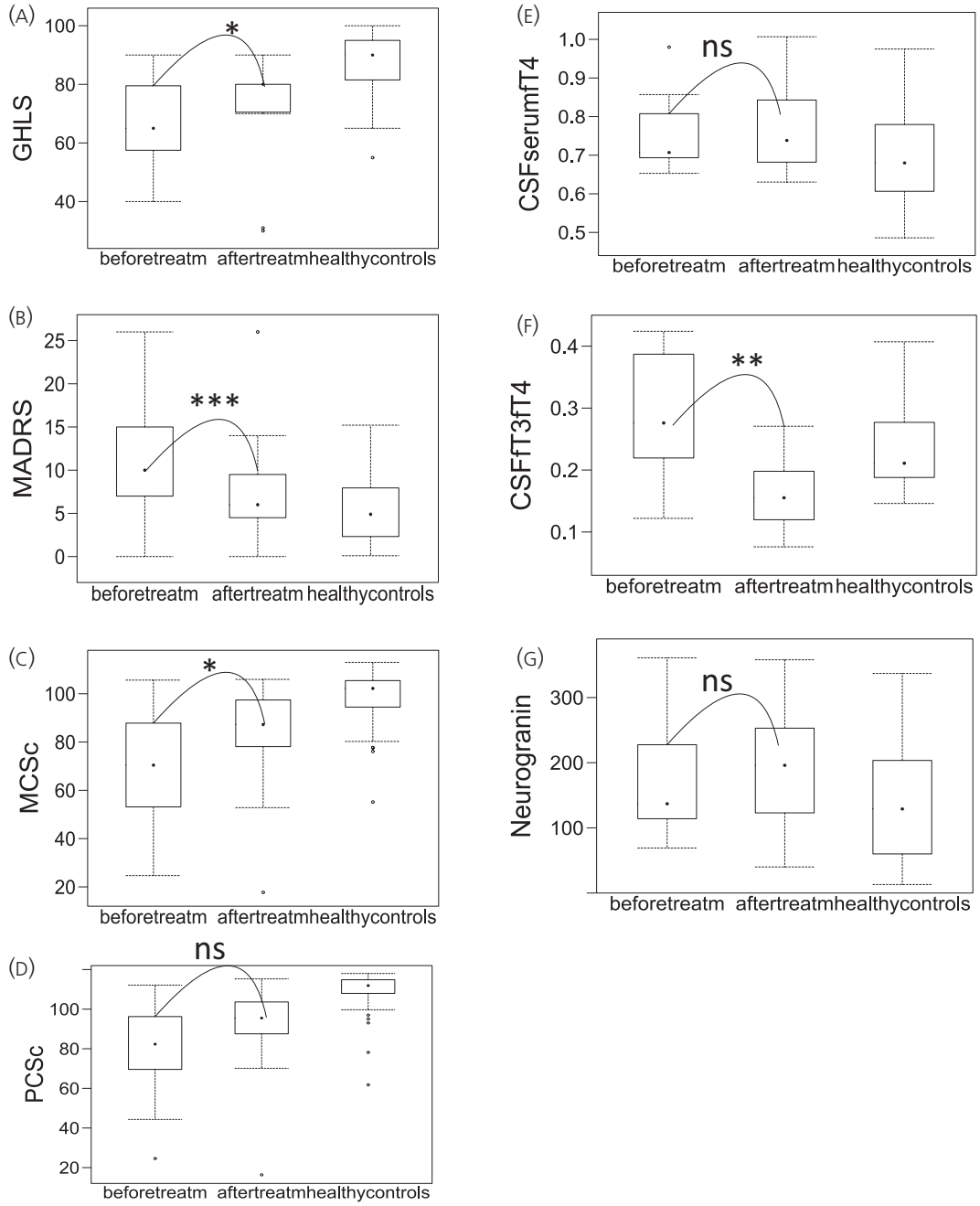

FIGURE 1 Results presented as box plots with median, interquartile range (IQR) and outliers. (A) Shows the Likert scale for general health (GHLS, points). (B) Illustrates the Montgomery-Åsberg Depression Rating Scale (MADRS) score (points) in PH before and during treatment. Since no data on MADRS in the healthy controls were collected in the present study, this data is based on a review article by Zimmerman et al. and values for 569 patients.[59] (C) Represents the mental subscore of SF-36 (MCSc, points). (D) Depicts the physical subscore of SF-36 (PCSc, points). (E) Shows the ratio between f-T4 in CSF and serum. (F) |Presents the ratio between f-T3 and f-T4 in CSF. Finally, box plot (G) illustrates neurogranin (pg/mL) in hypothyroid patients before treatment, hypothyroid patients during treatment and in healthy controls.

to increase (n.s.). However, during treatment, QoL was still lower in the patients compared with the healthy controls. In Table 2 data are also presented for 51 healthy controls.

3.2 | Thyroid hormones

During treatment, serum-TSH decreased from 8.5 to 0.8 mIU/L, and both serum and CSF f-T4 increased from 11.3 to 17.1 pmol/L and

TABLE 3 Spearman correlations between thyroid hormones, change in neurogranin and quality of life (QoL).

Spearman correlations	CSF/s f-T4 ratio during treatment (power of 10 transformation)	Change in neurogranin (during-before) (power of 10 transformation)
CSF/s f-T4 ratio during treatment		$r = -0.75$, $p = .0013$
Difference CSF/s f-T4 ratio (during–before) (power of 10 transformation)		$r = -0.68$, $p = .0048$
MADRS during treatment	$r = -0.65$, $p = .0081$	$r = 0.78$, $p = .0005$
Difference MADRS (during-before) (power of 10 transformation)	n.s. ($r = 0.49$, $p = .06$)	n.s. ($r = -0.45$, $p = .09$)
General Health Likert scale (GHLS) during treatment	$r = 0.52$, $p = .0494$	$r = -0.66$, $p = .0079$
General Health Likert scale (GHLS) difference (during-before) (power of 10 transformation)	n.s.	n.s.
Mental subscore SF-36 (MCSc) during treatment	$r = 0.80$, $p = .0003$	$r = -0.87$, $p = .00002$
Mental subscore SF-36 (MCSc) difference (during-before) (power of 10 transformation)	$r = 0.83$, $p = .0001$	$r = -0.86$, $p = .00003$
Physical subscore SF-36 (PCSc) during treatment	$r = 0.55$, $p = .0337$	$r = -0.64$, $p = .0109$
Physical subscore SF-36 (PCSc) difference (during-before) (power of 10 transformation)	$r = 0.61$, $p = .0164$	$r = -0.68$, $p = .0054$

from 8.5 to 13.1, respectively. Serum f-T3 increased from 5.4 to 6.3, but in CSF there were no significant changes.

There was also a significant decrease in both serum and CSF f-T3/f-T4 ratio, from 0.51 to 0.37 and 0.33 to 0.16 respectively (Table 2).

3.3 | Correlations between CSF/serum f-T4 ratio and QoL

The CSF/serum f-T4 ratio correlated strongest with QoL parameters during treatment, (Table 3 and Figure 2).

3.4 | Correlations between CSF/serum f-T3 ratio and QoL

There were no significant correlations between CSF/serum f-T3 ratio and QoL.

3.5 | Correlations between serum f-T3/f-T4 and QoL

The Spearman correlation test did not show any significant correlation between f-T3/f-T4 ratio in serum and QoL parameters.

3.6 | Correlations between CSF f-T3/f-T4 and QoL

The CSF f-T3/f-T4 ratio did not correlate significantly with any QoL parameters. Since treatment with L-thyroxine lowers the ratio between f-T3/f-T4 in both serum and CSF we also tested for correlations between ΔCSF f-T3/f-T4 and QoL parameters, but no significant correlation was found.

3.7 | Multiple comparison effect

The most important measurement we have for QoL is MCSc. The adjusted *p*-values gave a significance for serum f-T3 during treatment (adjusted $p = .032$) and for the CSF/serum f-T4 ratio during treatment (adjusted $p = .0024$) compared with MCSc during treatment. Other TH values and ratios were not significant to MCSc when adjusted.

3.8 | Neurogranin

The CSF concentration of neurogranin (Ng) did not differ from that in the healthy controls, neither before nor during treatment (HC: 155.2 ± 165.1; BT: 236.5 ± 228.5; DT: 218.8 ± 162.1, see also Table 2).

3.9 | Correlations between neurogranin and CSF/serum f-T4 ratio

There was no significant correlation between Ng level and CSF/serum f-T4 ratio during treatment, but there was a highly significant correlation between ΔNg (the change in Ng level) and the ratio ($r = -0.75$, $p = .001$). There was no significant correlation between ΔNg and the ratio of CSF/serum fT4 before treatment.

3.10 | Correlation between neurogranin and QoL

ΔNg correlated with all QoL parameters during treatment (Table 3).

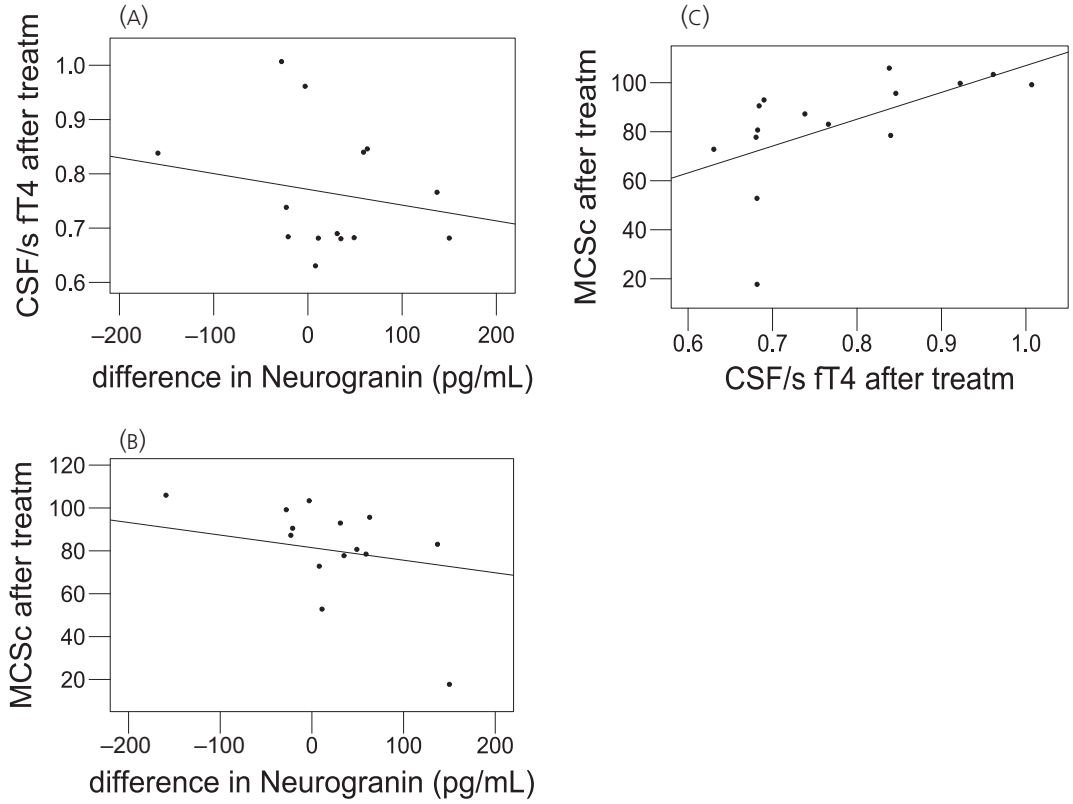

FIGURE 2 (A) Shows the correlation between the change in neurogranin (during–before, pg/mL) and the CSF/s f-T4 ratio during treatment ($r = -0.75$, $p = .001$). (B) Illustrates the correlation between the mental subscore of SF-36 (MCSc, points) during treatment and the change in neurogranin (during–before, pg/mL) ($r = -0.87$, $p < .0001$). (C) Depicts the correlation between the ratio between the free component of thyroxine (fT4) in CSF and serum during treatment and the mental subscore of SF-36 (MCSc, points) during treatment ($r = 0.80$, $p < .0005$).

4 | DISCUSSION

4.1 | The CSF/serum free T4 ratio

Measurements of the thyroid hormones f-T3 and f-T4 together with the pituitary hormone TSH in peripheral blood are widely used for monitoring L-thyroxine treatment. However, it has recently been reported that these hormone levels do not correlate with QoL measurements including mood parameters.[4,17] Earlier, only a few studies have investigated the relationship between serum and CSF levels of thyroid hormones,[34–36] but only to the extent of dynamics between the two compartments. We recently reported[22] that the CSF/serum f-T4 ratio, but not the serum or CSF concentrations of f-T4 or f-T3, was associated with QoL parameters, in untreated PH. The present study shows that also during L-thyroxine treatment, the CSF/serum f-T4 ratio is correlated with QoL estimates. No correlation was found for f-T3 or the CSF f-T3/f-T4 ratio, and not even for the ΔCSF f-T3/f-T4 ratio, which is regularly changed in response to treatment.[37] Furthermore, we believe that a very low QoL before treatment has a variety of different reasons. After L-thyroxine treatment, when our PH patients had a better general QoL, we observed that QoL measures were more markedly correlated with the CSF/serum f-T4 ratio despite the relatively small number of patients ($n = 15$).

4.2 | Thyroid hormones in CNS

All TH are produced in the thyrocytes of the thyroid gland. Under physiological normal pH conditions the main part of TH produced is T4.[38] There are some post synthetic conversions to T3 in the thyroid gland[39] but mainly T4 is the secreted hormone transported to the effector organs, for instance through the BBB. If T4 in CNS has a unique role for functions like mood is not fully clear although there are in vitro studies suggesting this.[40] Furthermore, mutations affecting any of the two most important proteins for the transport of T4 over the BBB results in severe mental retardation.[41,42] These two proteins, organic anion transporter1 C1 (OATP1c1) and monocarboxylate transporter 8 (MCT-8), have a higher affinity for T4 than for T3.[43,44] Therefore, the TH entering CNS is primarily T4, together with a minor amount of T3 passing via the MCT-8.[43,44] In addition to the entrance via these mechanisms there is evidence for a protein-binding transport from the choroid plexus to the CSF with, in the CNS existing protein, transthyretin (TTR).[45,46] The initial entrance to the choroid plexus is also via the TH transport proteins.[47] After the entrance, there is a local conversion inside the brain from T4 to T3. The activity of the DIOs, for this conversion, is regulated by TH meaning that when THs are abundant, the DIO2 activity is consequently downregulated. The DIO2 and the DIO3 are the

clinically active DIOs in the adult brain where the DIO3 exists as a scavenger mechanism in the neurons.[47,48]

The next step for the TH is to bind to the thyroid hormone receptors (TRs). Then, specific effects are induced in the CNS such as promoting axonal myelinization, dendritic spine growth, astrocyte and oligodendrocyte activation and microglial development.[49] Probably T3 mostly bind to TRs although T4 may also have some effect per se.[40] Thus, it is most likely that T3 is the main effector for the changes in gene transcription and other effects. It may therefore be surprising that we did not detect any correlation between QoL and the absolute concentration of f-T3 in CSF, or with the CSF/serum fT3 or CSF fT3/CSF fT4 ratios. Instead, we found a strong correlation with the CSF/serum f-T4 ratio.

It could be assumed from our results that low QoL, and other CNS symptoms in PH, are linked to deficiency of "T4-substrate" inside the brain. Therefore, the primary aim of treatment should be to activate or induce more abundant TH transport proteins. Another interesting theory regarding the "TH-substrate" for the brain is the TH-binding protein in the CNS, TTR. We have not measured the amount of this protein and, to our knowledge, no one has in hypothyroid patients. There are mouse studies with a knockout of TTR, and consequently lower amount of T4 in the CSF, and with developmental failures but no evidence that TTR plays a major role in grown up animals.[45] A recent review article for CSF biomarkers in depression[50] depict two studies, a total of 39 depressive patients, where TTR is lowered, compared to healthy controls. Whether this has any implication for the TH in the brain remains unknown. It seems most likely that TTR-status in humans is correlated with inflammation and malnutrition, and not with hypothyroidism.[51]

An important objective to the theory of elevated T4 as a primary goal is that in the present study CSF f-T4 concentration was increased by the L-thyroxine treatment, but there was no significant correlation between CSF f-T4 and the QoL measurements.

Therefore, it is probably not that simple–that a higher concentration of f-T4 in the brain is the most important. Instead, the truth probably lies in a more complex "TH-world." Therefore, the CSF/serum fT4 ratio could be a "marker" rather than an actual exerting mechanism. Although this "marker" of a better QoL in PH is undoubtedly controlled by the TH-transporters, the mechanisms underlying the effects on the TH-transporters could be of greater importance. It seems reasonable to believe that the main effector of the CNS symptoms in PH, that exerts an effect on great emotional and cognitive domains, is TRH. TRH is shown in several studies to be a widely CNS controlling hormone[52–55] and has even been proposed to be considered a neurotransmitter instead of a hormone.[56] It exerts effects on almost all hypothalamic systems[48,52–54,56,57] and is therefore not solely a hormone controlling the pituitary hormone TSH.

4.3 | Neurogranin

QoL parameters did not only correlate with the CSF/serum f-T4 ratio, but also with the ΔNg level, probably reflecting the degree of synaptic plasticity.[24,25] Ng is a protein kinase C substrate that binds calmodulin in its nonphosphorylated state. It is of great importance to transfer the signal in the postsynaptic neuron that begins with glutamate binding to the NMDA receptor. The building up of synapses from one neurons axon, or cell body, to other neurons dendrites is one of the most plastic processes in our body. If the brain is active in all its cognitive and emotional domains, we can grow several thousand synapses per day. In a more redundant stage, many of these synapses actually break down again, and some of the materials will be recycled elsewhere. When synapses degrade or remodel, there is leakage or secretion of, the whole or especially the C-terminal domain of the Ng protein. This protein can be analyzed in CSF and is believed to reflect postsynaptic dendritic plasticity. Xiang et al.[26] have also suggested that Ng can be used as a biomarker in a variety of diseases affecting the brain, such as neurodegenerative, neurological and psychiatric diseases. In experimental studies, knockout of the Ng-gene in mice caused disability to build this protein, leading to impaired spatial memory.[23,24] Interestingly, administration of L-thyroxine can, in part, reverse this impairment.[24] The transcription of the Ng-gene is controlled by a variety of factors, including TH. The Ng-gene was the first gene in the brain that was found to be regulated by THs.[24]

It has been proposed that Ng is a valid marker of the effects of THs in the rodent CNS.[49] As Ng is important for neuronal plasticity, we hypothesized that Ng would be decreased during L-thyroxine treatment in PH, but in the present study the CSF Ng level nonsignificantly tended to increase during treatment (Figure 1). Thus, these findings do not support our hypothesis that Ng is a marker of brain insufficiency in PH. However, ΔNg (the change in Ng level) correlated significantly with all QoL parameters during treatment, with lower QoL after L-thyroxine treatment in patients who had marked increases in Ng concentrations (Figure 2). Ng-levels in CSF might be a unique biomarker of synaptic plasticity that could be used in further research on diseases affecting cognition, even in mild cases. The changes in Ng-levels must always be looked at on an individual, not on a group level.

An elevation of Ng on the other hand, from within a reference interval, may reflect a disease that primarily degrade neurons, that is, a neurodegenerative disease. A recent review of biomarkers in depression[50] did not find any changes in absolute concentration of Ng, in two studies comparing Ng in depression from healthy controls. Because Ng levels in PH patients were in close proximity as to the levels in the healthy controls, and the changes in CSF Ng levels were variable in individual PH patients, we cannot state that PH is a neurodegenerative disease affecting the degradation of neurons. Since there was an increase in Ng in about half of the patients and the other half decreased we cannot state that TH in the brain is directly involved in Ng dynamics (meaning transcription and degradation of Ng). Instead, as PH clearly does change cognitive processing, we believe that this change is seen in the Ng measurements of CSF. Therefore, being a marker for synaptic plasticity,[25,58] the Ng concentration in CSF would presumably be changed in any disease affecting cognitive processes, and not only because of concentrations of TH in the CNS.

4.4 | Practical implications

In summary, we found that CSF/serum f-T4 ratio, but not fT3 or f-T4 concentrations in serum and CSF, is strongly correlated with QoL parameters during L-thyroxine treatment, which to our knowledge has not been reported previously. Our group of patients had a TSH level of mean 8.5, meaning they would be considered to have a mild hypothyroidism and this correlation still exists. During treatment of PH, it must be considered that serum levels of thyroid hormones do not mirror the CSF levels. An important question is whether it is clinically useful to perform a lumbar puncture in hypothyroid patients suffering from severe residual symptoms. A precondition for this procedure in routine clinical practice is that the CSF/serum f-T4 ratio can provide useful information for the management of the individual patients including dose titration. Thus, further research studies are needed to evaluate the potential role of the CSF/serum f-T4 ratio and therefore we cannot yet recommend it in a clinical setting. Note the last of our final take home messages for the clinician treating hypothyroid patients is to always be aware that the disease and first-line treatment might not normalize the dynamics of TH inside the brain, even if measurements in peripheral blood are changed in the direction of healthy persons levels.

4.5 | Limitations of the study

One major limitation of our study was the low number of patients. We had a dropout rate of 40% from our original PH population. This relatively high percentage of patients not coming to the second visit during treatment was probably, to a great extent, associated with the discomfort experienced by patients having to undergo a second lumbar puncture. It is difficult to motivate patients to come for two subsequent lumbar punctures, where there is no obvious benefit for the individual patient. However, as the present study shows that the CSF/serum f-T4 ratio can provide additional information, it may be easier to motivate patients with residual hypothyroid symptoms to participate in future studies.

AUTHOR CONTRIBUTIONS

Anders Funkquist: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; validation; visualization; writing – original draft. **Birger Wandt:** Writing – review and editing. **Kaj Blennow:** Resources; writing – review and editing. **Henrik Zetterberg:** Resources; writing – review and editing. **Johan Svensson:** Writing – review and editing. **Per Bjellerup:** Resources; writing – review and editing. **Yvonne Freund-Levi:** Writing – review and editing. **Stefan Sjöberg:** Conceptualization; data curation; formal analysis; investigation; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest is reported.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

An excel document of primary data will be available on request.

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REFERENCES

1. Lerner A, Jeremias P, Matthias T. The world incidence and prevalence of autoimmune diseases is increasing. *Int J Celiac Dis*. 2015;3(4):151-155.
2. Statistical Database. The National board of health and welfare statistical database on pharmaceutical products. http://www.socialstyrelsen.se/statistik/statistikdatabas/lakemedel
3. Hogervorst E, Huppert F, Matthews FE, Brayne C. Thyroid function and cognitive decline in the MRC cognitive function and ageing study. *Psychoneuroendocrinology*. 2008;33(7):1013-1022.
4. Samuels MH, Kolobova I, Niederhausen M, Janowsky JS, Schuff KG. Effects of altering levothyroxine (L-T4) doses on quality of life, mood, and cognition in L-T4 treated subjects. *J Clin Endocrinol Metab*. 2018;103(5):1997-2008.
5. Forman-Hoffman V, Philibert RA. Lower TSH and higher T4 levels are associated with current depressive syndrome in young adults. *Acta Psychiatr Scand*. 2006;114(2):132-139.
6. Kong SH, Kim JH, Park YJ, et al. Low free T3 to free T4 ratio was associated with low muscle mass and impaired physical performance in community-dwelling aged population. *Osteoporos Int*. 2020;31(3):525-531.
7. Eslami-Amirabadi M, Sajjadi SA. The relation between thyroid dysregulation and impaired cognition/behaviour: an integrative review. *J Neuroendocrinol*. 2021;33(3):e12948.
8. Chaker L, Bianco AC, Jonklaas J, Peeters RP. *Hypothyroidism*. Vol 390. Lancet; 2017:1550-1562.
9. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Intern Med*. 2000;160(4):526-534.
10. Jonklaas J, Bianco AC, Bauer AJ, et al. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid*. 2014;24(12):1670-1751.
11. Singh R, Tandon A, Gupta SK, Saroja K. Optimal levothyroxine replacement adequately improves symptoms of hypothyroidism; residual symptoms need further evaluation for other than hypothyroidism causation. *Indian J Endocrinol Metab*. 2017;21(6):830-835.
12. Mitchell AL, Hegedus L, Zarkovic M, Hickey JL, Perros P. Patient satisfaction and quality of life in hypothyroidism: an online survey by the british thyroid foundation. *Clin Endocrinol (Oxf)*. 2021;94(3):513-520.

13. Winther KH, Cramon P, Watt T, et al. Disease-specific as well as generic quality of life is widely impacted in autoimmune hypothyroidism and improves during the first six months of levothyroxine therapy. *PLoS One*. 2016;11(6):e0156925.
14. Reuters VS, Almeida Cde P, Teixeira Pde F, et al. Effects of subclinical hypothyroidism treatment on psychiatric symptoms, muscular complaints, and quality of life. *Arq Bras Endocrinol Metabol*. 2012;56(2):128-136.
15. Bianchi GP, Zaccheroni V, Solaroli E, et al. Health-related quality of life in patients with thyroid disorders. *Qual Life Res*. 2004;13(1):45-54.
16. Magner J. Historical note: many steps led to the 'discovery' of thyroid-stimulating hormone. *Eur Thyroid J*. 2014;3(2):95-100.
17. Fitzgerald SP, Bean NG, Hennessey JV, Falhammar H. Thyroid testing paradigm switch from thyrotropin to thyroid hormones-future directions and opportunities in clinical medicine and research. *Endocrine*. 2021;74(2):285-289.
18. Williams GR, Bassett JH. Deiodinases: the balance of thyroid hormone: local control of thyroid hormone action: role of type 2 deiodinase. *J Endocrinol*. 2011;209(3):261-272.
19. Gullo D, Latina A, Frasca F, Le Moli R, Pellegriti G, Vigneri R. Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One*. 2011;6(8):e22552.
20. Ettleson MD, Bianco AC. Individualized therapy for hypothyroidism: is T4 enough for everyone? *J Clin Endocrinol Metab*. 2020;105(9):e3090-e3104.
21. Leonard JL, Siegrist-Kaiser CA, Zuckerman CJ. Regulation of type II iodothyronine 5′-deiodinase by thyroid hormone. Inhibition of Actin polymerization blocks enzyme inactivation in cAMP-stimulated glial cells. *J Biol Chem*. 1990;265(2):940-946.
22. Funkquist A, Bengtsson A, Johansson PM, et al. Low CSF/serum ratio of free T4 is associated with decreased quality of life in mild hypothyroidism-a pilot study. *J Clin Transl Endocrinol*. 2020;19:100218.
23. Bernal J. Thyroid Hormones and Brain Development. 2009.
24. Bernal J. Thyroid hormone receptors in brain development and function. *Nat Clin Pract Endocrinol Metab*. 2007;3(3):249-259.
25. Pak JH, Huang FL, Li J, et al. Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: a study with knockout mice. *Proc Natl Acad Sci U S A*. 2000;97(21):11232-11237.
26. Xiang Y, Xin J, Le W, Yang Y. Neurogranin: a potential biomarker of neurological and mental diseases. *Front Aging Neurosci*. 2020;12:584743.
27. Zulewski H, Muller B, Exer P, Miserez AR, Staub JJ. Estimation of tissue hypothyroidism by a new clinical score: evaluation of patients with various grades of hypothyroidism and controls. *J Clin Endocrinol Metab*. 1997;82(3):771-776.
28. Beever K, Bradbury J, Phillips D, et al. Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. *Clin Chem*. 1989;35(9):1949-1954.
29. Portelius E, Olsson B, Hoglund K, et al. Cerebrospinal fluid neurogranin concentration in neurodegeneration: relation to clinical phenotypes and neuropathology. *Acta Neuropathol*. 2018;136(3):363-376.
30. Gudex C, Dolan P, Kind P, Williams A. Health state valuations from the general public using the visual analogue scale. *Qual Life Res*. 1996;5(6):521-531.
31. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *The British Journal of Psychiatry: The Journal of Mental Science*. 1979;134:382-389.
32. Taft C, Karlsson J, Sullivan M. Performance of the Swedish SF-36 version 2.0. *Qual Life Res*. 2004;13(1):251-256.
33. Farivar SS, Cunningham WE, Hays RD. Correlated physical and mental health summary scores for the SF-36 and SF-12 health survey, V.I. *Health Qual Life Outcomes*. 2007;5:54.
34. Hansen JM, Siersbaek-Nielsen K. Cerebrospinal fluid thyroxine. *J Clin Endocrinol Metabol*. 1969;29(8):1023-1026.
35. Burman P, Hetta J, Wide L, Månsson JE, Ekman R, Karlsson FA. Growth hormone treatment affects brain neurotransmitters and thyroxine. *Clin Endocrinol (Oxf)*. 1996;44(3):319-324.
36. Sjoberg S, Eriksson M, Nordin C. L-thyroxine treatment and neurotransmitter levels in the cerebrospinal fluid of hypothyroid patients: a pilot study. *Eur J Endocrinol*. 1998;139(5):493-497.
37. Sjoberg S, Eriksson M, Werner S, Bjellerup P, Nordin C. L-thyroxine treatment in primary hypothyroidism does not increase the content of free triiodothyronine in cerebrospinal fluid: a pilot study. *Scand J Clin Lab Invest*. 2011;71(1):63-67.
38. de Vijlder JJ, den Hartog MT. Anionic iodotyrosine residues are required for iodothyronine synthesis. *Eur J Endocrinol*. 1998;138(2):227-231.
39. Carvalho DP, Dupuy C. Thyroid hormone biosynthesis and release. *Mol Cell Endocrinol*. 2017;458:6-15.
40. Schroeder AC, Privalsky ML. Thyroid hormones, t3 and t4, in the brain. *Front Endocrinol (Lausanne)*. 2014;5:40.
41. Allan W, Herndon CN, Dudley FC. Some examples of the inheritance of mental deficiency: apparently sex-linked idiocy and microcephaly. *Am J Ment Defic*. 1944;48:325-334.
42. Stromme P, Groeneweg S, Lima de Souza EC, et al. Mutated thyroid hormone transporter OATP1C1 associates with severe brain Hypometabolism and juvenile neurodegeneration. *Thyroid*. 2018;28(11):1406-1415.
43. Suzuki T, Abe T. Thyroid hormone transporters in the brain. *Cerebellum*. 2008;7(1):75-83.
44. Kakinuma H, Itoh M, Takahashi H. A novel mutation in the monocarboxylate transporter 8 gene in a boy with putamen lesions and low free T4 levels in cerebrospinal fluid. *J Pediatr*. 2005;147(4):552-554.
45. Richardson SJ, Wijayagunaratne RC, D'Souza DG, Darras VM, Van Herck SL. Transport of thyroid hormones via the choroid plexus into the brain: the roles of transthyretin and thyroid hormone transmembrane transporters. *Front Neurosci*. 2015;9:66.
46. Wirth EK, Schweizer U, Kohrle J. Transport of thyroid hormone in brain. *Front Endocrinol (Lausanne)*. 2014;5:98.
47. Bianco AC, Dumitrescu A, Gereben B, et al. Paradigms of dynamic control of thyroid hormone signaling. *Endocr Rev*. 2019;40(4):1000-1047.
48. Heuer H. The importance of thyroid hormone transporters for brain development and function. *Best Pract Res Clin Endocrinol Metab*. 2007;21(2):265-276.
49. Noda M. Thyroid hormone in the CNS: contribution of neuron-glia interaction. *Vitam Horm*. 2018;106:313-331.
50. Mousten IV, Sorensen NV, Christensen RHB, Benros ME. Cerebrospinal fluid biomarkers in patients with unipolar depression compared with healthy control individuals: a systematic review and meta-analysis. *JAMA Psychiat*. 2022;79(6):571-581.
51. Carriere I, Dupuy AM, Lacroux A, Cristol JP, Delcourt C. Pathologies Oculaires Liees a l'Age study G. biomarkers of inflammation and malnutrition associated with early death in healthy elderly people. *J Am Geriatr Soc*. 2008;56(5):840-846.
52. Broberger C, McCormick DA. Excitatory effects of thyrotropin-releasing hormone in the thalamus. *J Neurosci*. 2005;25(7):1664-1673.
53. Gonzalez JA, Horjales-Araujo E, Fugger L, Broberger C, Burdakov D. Stimulation of orexin/hypocretin neurones by thyrotropin-releasing hormone. *J Physiol*. 2009;587(Pt 6):1179-1186.
54. Horjales-Araujo E, Hellysaz A, Broberger C. Lateral hypothalamic thyrotropin-releasing hormone neurons: distribution and relationship to histochemically defined cell populations in the rat. *Neuroscience*. 2014;277:87-102.

55. Hara J, Gerashchenko D, Wisor JP, Sakurai T, Xie X, Kilduff TS. Thyrotropin-releasing hormone increases behavioral arousal through modulation of hypocretin/orexin neurons. *J Neurosci*. 2009;29(12):3705-3714.
56. Frohlich E, Wahl R. The forgotten effects of thyrotropin-releasing hormone: metabolic functions and medical applications. *Front Neuroendocrinol*. 2019;52:29-43.
57. Lechan RM, Fekete C. The TRH neuron: a hypothalamic integrator of energy metabolism. *Prog Brain Res*. 2006;153:209-235.
58. Zhong L, Gerges NZ. Neurogranin Regulates Metaplasticity. *Front Mol Neurosci*. 2019;12:322.
59. Zimmerman M, Chelminski I, Posternak M. A review of studies of the Montgomery-Asberg depression rating scale in controls: implications for the definition of remission in treatment studies of depression. *Int Clin Psychopharmacol*. 2004;19(1):1-7.

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