RESEARCH

The Role of Thyroid Hormone Synthesis Gene-Related miRNAs Profiling in Structural and Functional Changes of The Thyroid Gland Induced by Excess Iodine

Chunyu Zhang[1] · Jinyin Yao[2] · Chang Liu[1] · Kunying Yang[1] · Wei Zhang[1] · Dianjun Sun[1] · Weikuan Gu[3]

Abstract

At recent years, the impairment caused by iodine excess are paid more attention. However, there is still largely unknown about the exact mechanism induced by excessive iodine. MiRNAs have been found to act as biomarkers for a variety of diseases, whereas fewer studies focused on miRNAs related to a cluster of genes regulating thyroid hormone synthesis, such as NIS, Pendrin, TPO, MCT8, TSHR, TSHα, and TSHβ-related miRNAs in structural and functional changes of the thyroid gland induced by subchronic and chronic high iodine exposure. In the present study, one hundred and twenty 4-week-old female Wistar rats were randomly divided into control group ($I_{50μg/L}$ KIO$_3$); HI 1 ($I_{6000μg/L}$ KIO$_3$); HI 2 ($I_{10000μg/L}$ KIO$_3$); and HI 3 ($I_{50000μg/L}$ KIO$_3$), the exposure period was 3 months and 6 months, respectively. The iodine contents in the urine and blood, thyroid function, and pathological changes were determined. In addition, levels of thyroid hormone synthesis genes and the associated miRNAs profiling were detected. The results showed that subclinical hypothyroidism occurred in the high iodine groups with subchronic high iodine exposure, while 6-month exposure led to hypothyroidism in the $I_{10000μg/L}$ and $I_{50000μg/L}$ groups. Subchronic and chronic high iodine exposure caused mRNA and protein levels of NIS, TPO, and TSHR decreased significantly, and Pendrin expression increased significantly. In addition, MCT8 mRNA and protein levels are only remarkably decreased under the subchronic exposure. PCR results showed that levels of miR-200b-3p, miR-185-5p, miR-24-3p, miR-200a-3p, and miR-25-3p increased significantly exposed to high iodine for 3 months, while miR-675-5p, miR-883-5p, and miR-300-3p levels increased significantly under the exposure to high iodine for 6 months. In addition, miR-1839-3p level was markedly decreased exposed to high iodine for 3 and 6 months. Taken together, the miRNA profiling of genes regulating thyroid hormone synthesis remarkably altered from subclinical hypothyroidism to hypothyroidism induced by excess iodine exposure, and some miRNAs may play an important role in subclinical hypothyroidism or hypothyroidism through regulating NIS, Pendrin, TPO, MCT8, and TSHR providing promising targets to alleviate the impairment on the structure and function of thyroid gland.

Keywords Iodine excess · Subclinical hypothyroidism · Hypothyroidism · Goiter · miRNA

Introduction

Iodine is an essential micronutrient for normal physiological activities, both iodine deficiency and iodine excess are harmful to human health [1]. Hypothyroidism, hyperthyroidism, thyroid cancer, autoimmune thyroid disease, and other thyroid disorders can occur when iodine intake is excess [2]. At recent years, the diseases caused by iodine excess are paid more attention. Excess iodine is mainly ingested from the high concentration of iodine in drinking water, and China has the largest area of high iodine in the world [3]. However, there is still largely unknown about the exact mechanism induced by excessive iodine on the human health.

When iodine intake is excessive, the thyroid gland can maintain thyroid hormone levels through a variety of control mechanisms, such as TSH, the sodium/iodide symporter (NIS), The Wolff-Chaikoff effect, redistribution of organic iodine, non organic iodine redistribution, and non-organic iodine secretion. Iodine ions are taken in from the blood mainly through NIS located in

Chunyu Zhang and Jinyin Yao have contributed equally to this work and share first authorship.

Extended author information available on the last page of the article

the basolateral membrane and transported to the follicular glia via the Pendrin protein on the free rim membrane, where iodine ions are activated by hydrogen peroxide under the action of thyroid peroxidase (TPO), and synthesized T4 and T3, which are then released into the blood via the monocarboxylate transporter 8 (MCT8) at the cell base to exert physiological effects. Thyroid hormone secretion is also regulated by the hypothalamic-pituitary-thyroid axis system, TSHβ, and TSHα secreted by the pituitary gland directly, and act on downstream genes through binding to the thyroid stimulating hormone receptor (TSHR) to promote the synthesis and secretion of thyroid hormone. It has been shown that thyroid damage caused by excessive iodine is mainly due to the expression inhibition of iodine metabolism-related genes, such as NIS, TPO, MCT8, and TSHR [4–6] and do harm to thyroid hormone synthesis and secretion. Nevertheless, few studies on the genes are related to thyroid hormone synthesis in pituitary-thyroid axis, such as TSHα and TSHβ.

MicroRNAs (miRNAs) are small non-coding ribonucleic acids (ncRNAs) which are highly conserved among species, with 18–24 nucleotides in length, leading to translational repression or gene degradation through binding to complementary sequences in the 3' untranslated region (UTR) of target genes [7]. MiRNAs are widely involved in physiological and pathological processes of the organism, such as cancer, inflammatory diseases, and metabolic disorders, used as biomarkers and therapeutic targets [8]. Previous study found that miRNAs are differentially expressed between patients with thyroid cancer and healthy individuals, in vivo and in vitro studies suggest that miRNA dysregulation plays an important role in the progression of thyroid cancer, such as miR-7 [9], miR-141-3p [10], miR-144 [11], miR-524-5p [12], miR-150 [13], and miR-199b-5p [14]. Moreover, miRNAs have an effect on hypothyroidism. Liu et al. found that miR-15b-5p could inhibit hypothyroidism by targeting and regulating Stat3 expression in HypoT rat brain [15]; and Li WJ et al. suggested that miRNA-124-3p level reduced in the hippocampus of pregnant rats between day 15 of gestation and delivery, thus prevented congenital hypothyroidism by targeting programmed cell death protein 6 [16]. Hebatallah HA et al. found that high levels of miR-224-5p could play a role in hypothyroidism-induced thyroid injury through downregulation of D1 [17].

Taken together, the present study was conducted to establish subchronic and chronic high water iodine exposure rat models, detect the levels of a series of thyroid hormone synthesis genes NIS, Pendrin, TPO, MCT8, TSHR, TSHα, TSHβ, and related miRNA profiles to find promising targets to alleviate the harm of excess iodine exposure.

Materials and Methods

Animals and Treatments

A total of one hundred and twenty 4-week-old SPF-grade Wistar female rats (90–110 g) were purchased from Beijing Viton Lihua Laboratory Animal Technology Co. The rats were housed with an ambient temperature of 20 ± 2 °C, relative humidity of 40–80%, and 12 h of light/darkness cycle, and were fed with water ad libitum. After 1 week of acclimatization, the rats were randomly divided into 4 group (each group has 30 rats) according to the body weight. Based on previous research and findings of our group, each group was treated as follows: control group (basic diet, drinking deionized water + KIO_3 solution containing iodine ions at 50 µg/L); $I_{6000 \text{ µg/L}}$ group (basic diet, drinking deionized water + KIO_3 solution containing iodine ions at 6000 µg/L); $I_{10000 \text{ µg/L}}$ group (basic diet, drinking deionized water + KIO_3 solution containing iodine ions at 10,000 µg/L); $I_{50000 \text{ µg/L}}$ group (basic feed, drinking deionized water + KIO_3 solution containing iodine ions 50,000 µg/L). The feed given to rats is clean grade rat maintenance feed, the manufacturer is Beijing Co-operative Feed Co. The experimental protocol was approved by the Animal Ethics Committee of Harbin Medical University.

Collection of Biological Samples

Thyroid Gland Collection Both lateral thyroid glands were peeled off, weighed, the left thyroid gland was placed in a 1.5ml centrifuge tube and frozen in liquid nitrogen for 15 min and then placed in a $-80℃$ refrigerator, and the right thyroid tissue was fixed in 4.0% paraformaldehyde at room temperature for H&E staining and immunohistochemistry.

Pituitary Gland Collection The pituitary gland was collected, washed in saline, weighed, and part of it was fixed in 4.0% paraformaldehyde solution and sectioned by conventional paraffin embedding for immunohistochemistry.

Blood and Urine Iodine Determination

The rats were placed in metabolic cage, and 24-h urine was collected by a 15 ml centrifuge tube. After collection, let it stand, centrifuge at 3000 rpm for 10 min, then pour it into a 5 ml centrifuge tube and store it in a refrigerator at $-20℃$. Urine iodine was detected by arsenic cerium catalytic spectrophotometry (Health Industry standard of the People's Republic of China WS/T 107.1–2016).

Rats were anesthetized by intraperitoneal injection of 2.5% avodin. After the rats were completely anesthetized, approximately 10 ml of blood was taken from the abdominal aorta. Among them, 5 ml is placed in an anticoagulant blood collection tube containing heparin sodium, and 5 ml is placed in a blood collection tube without anticoagulant. After standing for about 2 h, the upper layer of serum or plasma is subpackaged and labeled, and stored at $-80℃$ to detect blood iodine by Arsenic-Cerium Catalytic Spectrophotometry (WS/T572-2017).

Determination of Thyroid Function and Antibodies

The levels of TgAb, TPOAb, FT3, and FT4 in serum were measured using radioimmunoassay kits by taking 10 µL, 100 µL, 50 µL, and 50 µL of serum, respectively, according to the manufacturer's instructions, and measuring the radioactivity count in each precipitation tube. TSH levels were determined using an ELISA kit by taking 50 µL of serum.

H&E Staining

Tissue from 4.0% paraformaldehyde was rinsed in tap water, dehydrated in a gradient of ethanol, clear in xylene, dipped in wax, then embedded, and sectioned 4 µm thick using a microtome (Leica, Germany). The sections were dewaxed in xylene, dehydrated in a high percentage ethanol (from 70 to 100%) gradient, hematoxylin-stained nuclei for 15 min, rinsed with tap water to reverse the blue, eosin-stained for 1 min, dehydrated and transparent, and sealed with neutral resin. Histopathological changes of the thyroid gland were observed using BX53 light microscope (Olympus, Japan).

Immunohistochemistry Staining

After dewaxing and dehydrating the sections transparently, 3% hydrogen peroxide was used to inactivate peroxidase, followed by antigen repair with sodium citrate (pH = 6.0) and blocked with 10% BSA at room temperature for 30 min. Then, the specific antibodies NIS (1:150 Origene, #FA15aa1025), Pendrin (1:25, Invitrogen, #AA4A04N), TPO (1:50, Abcam, #GR3271630-4), MCT8 (1:150, abcam, #ab214446), TSHR (1:150, Abcam, GR3238700-2), TSHα (1:150, LsBio, #174,432), and TSHβ (1:50, NovusBio, #A118620) incubated overnight at 4 °C, followed by a rabbit two-step kit (Nakasugi Golden Bridge, PV-6001) at 37℃ for 30 min, and hematoxylin-stained nuclei were then developed using a DAB color development kit (Nakasugi Golden Bridge, ZLI-9018). Images were photoed by BX53 light microscope (Olympus, Japan), and analyzed by Image-J software (National Institutes of Health, USA).

Quantitative Real-time PCR Analysis

Briefly, total RNA and miRNA were extracted from thyroid and pituitary tissues using TRIzol reagent (Takara, Japan) and then reversely transcribed into cDNA using the PrimeScript RT kit (Takara) and Mir-X miRNA First-strand kit (Takara, Japan) according to standard protocols, respectively. The mRNA and miRNA expression levels were detected using SYBR PrimeScript RT-PCR Kit II (Takara, Japan) and QPCR Systems (QuantStudio 5, USA). Relative expressions of mRNA and miRNA levels were analyzed using the $2^{-\triangle\triangle CT}$ method after normalization to β-actin and U6, respectively. Primer information are shown in Table 1.

Table 1 Primer information

Gene	Primer sequence (5'-3')
Slc5a5-F	AGCCTCGCTCAGAACCATTC
Slc5a5-R	GTGTACCGGCTCCGAGGAT
MCT8-F	AGGCCTTGGACGTCTCGTCT
MCT8-R	GACATCAAGCCCAGGAGCAG
TPO-F	CTACTGTGTCCAACGTCTTCTC
TPO-R	GGTCCTGGAAGTCTGTGTTTAG
TSHR-F	TCGAGCCTGCCCAATATTTC
TSHR-R	CTGGTGTTCCGGATTTCTATGT
Slc26a4-F	CTCATTCAAGACTGCAAAGATCCTC
Slc26a4-R	CTAGCAGTCCTCGCTGACCAA
CGA-F	ACGTGCTGTGGCCAAATC
CGA-R	TGGCACATGGAAGCTACGA
TSHb-F	CATCTGCGCTGGGTATTGTATGA
TSHb-R	AGCAACATGGTGTGGGCATC
β-actin-F	GCAGATGTGGATCAGCAAGC
β-actin-R	GGTGTAAAACGCAGCTCAGTAA
rno-miR-200b-3p	TAATACTGCCTGGTAATGATGAC
rno-miR-24-3p	TGGCTCAGTTCAGCAGGAACAG
rno-miR-185-5p	TGGAGAGAAAGGCAGTTCCTGA
rno-miR-1839-3p	AGACCTACTTATCTACCAACAG
rno-miR-200a-3p	TAACACTGTCTGGTAACGATGT
rno-miR-25-3p	CATTGCACTTGTCTCGGTCTGA
rno-miR-675-5p	TGGTGCGGAAAGGGCCCACAGT
rno-miR-883-5p	TGCTGAGAGAAGTAGCAGTTACT
rno-miR-300-3p	TATGCAAGGGCAAGCTCTCTTC
U6-F	GGAACGATACAGAGAAGATTAGC
U6-R	TGGAACGCTTCACGAATTTGCG

In silico *Analysis*

To find miRNAs regulating NIS, Pendrin, TPO, MCT8, and TSHR, we performed computer analysis in three independent databases: miRDB (http://mirdb.org/mirdb/index.html), miRWalk (http://mirwalk.umm.uniheidelberg.de/), and TargetScan (https://www.targetscan.org/vert_72/), and miR-200b-3p, miR-185-5p, miR-24-3p, miR-200a-3p, miR-25-3p, miR-675-5p, miR-883-5p, miR-300-3p, and miR-1839-3p were found byprediction.

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Inc, USA) and SPSS 25.0 (SPSS Software Inc, USA). One-way ANOVA was used for quantitative variables among different high iodine groups, LSD or Tamhane T2 was used for Post Hoc Comparison. Pearson correlation (for the data conforms to the normal distribution) and Spearman's rank correlation (for nonnormal distribution data) were used for bivariate correlation analysis. $P < 0.05$ as a difference of statistical significance.

Results

The Effects of Subchronic and Chronic High Water Iodine Exposure on Thyroid Structure and Function

Compared with the control group, levels of urinary iodine (Fig. 1A) and blood iodine (Fig. 1B) were remarkably higher in the $I_{6000 \mu g/L}$, $I_{10000 \mu g/L}$, and $I_{50000 \mu g/L}$ groups after 3 and 6 months' exposure, and increased with the increasing doses of iodine, showing a dose–response relationship ($P < 0.05$). These indicated that the animal model of Wistar rats with subchronic and chronic high iodine exposure was successfully established.

TSH levels in the serum in 3-month $I_{6000 \mu g/L}$, $I_{10000 \mu g/L}$, and $I_{50000 \mu g/L}$ groups were significantly higher than that in the control group (Fig. 1C). Compared with the control group, no significant changes found in FT3, FT4, TPOAb, and TGAb levels, except for the significant decrease in TGAb levels in the $I_{6000 \mu g/L}$ group (Fig. 1D–G). After 6 months' exposure, serum TSH levels in the $I_{6000 \mu g/L}$, $I_{10000 \mu g/L}$, and $I_{50000 \mu g/L}$ groups were significantly higher in comparison to the control group (Fig. 1C). Moreover, FT3 and FT4 levels were significantly lower in the $I_{10000 \mu g/L}$ and $I_{50000 \mu g/L}$ groups (Fig. 1D, E). However, there were no significant changes in TPOAb and TGAb levels in high iodine groups, except the level of TPOAb remarkably decreased in the $I_{10000 \mu g/L}$ group, compared with the control group (Fig. 1F, G). The results showed that subclinical hypothyroidism occurred in $I_{6000 \mu g/L}$, $I_{10000 \mu g/L}$, and $I_{50000 \mu g/L}$ groups after 3 months of high iodine exposure, and hypothyroidism occurred in $I_{10000 \mu g/L}$ and $I_{50000 \mu g/L}$ groups after 6 months' exposure.

H&E staining showed that the thyroid follicular epithelial cells were columnar or cuboidal, the nuclei were round, the follicular size was uniform, and there was a medium amount of pink glia in the lumen both in 3-month and 6-month control groups. In high iodine groups exposed for 3 or 6 months, thyroid follicular epithelial cells become flat, the nucleus is pike shaped, follicular cavity increases, follicular size differs, and follicular cavity is filled with a large number of deep dyed red gum. The degree of above changes increase with the increasing dose of iodine dose (Fig. 1K), and thyroid body ratio in 6-month $I_{10000 \mu g/L}$ and $I_{50000 \mu g/L}$ groups were significantly increased. In addition, no significant change found in the body weight under different high doses of iodine after subchronic or chronic exposure (Fig. 1H–J). The above results suggested that chronic high iodine exposure caused more severe goiter in $I_{10000 \mu g/L}$ and $I_{50000 \mu g/L}$ groups than subchronic iodine exposure.

The Effects of Excessive Iodine on mRNA and Protein Expression Levels of Thyroid Hormone Synthesis Genes

The mRNA and protein levels of NIS, Pendrin, TPO, MCT8, TSHR, TSHα, and TSHβ were subsequently detected, due to their crucial roles in the synthesis of thyroid hormones. Compared with the corresponding control group, the mRNA levels of NIS, TPO, and TSHR were significantly lower, Pendrin mRNA levels were significantly higher, while TSHα and TSHβ levels were not obviously changed in the high iodine group under 3 or 6 months' exposure. Moreover, the MCT8 mRNA levels were highly decreased after 3 months' high iodine exposure. However, no significant difference was found among the high iodine groups after 6 months' exposure. (Fig. 2A).

IHC staining revealed that NIS, Pendrin, TPO, MCT8, and TSHR proteins were mainly located in the cytoplasm of thyroid follicular epithelial cells. TSHα and TSHβ were mainly expressed in the cytoplasm of endocrine cells in pituitary gland. AOD values analysis showed that compared with the control group, the protein expression levels of NIS, TPO, and TSHR were significantly decreased in the high-iodine groups (Fig. 2B, D, E), while the protein expression levels of Pendrin were significantly increased (Fig. 2F). Except for the increased protein levels of TSHα in the $I_{50,000 ug/L}$ group under 3-month exposure, TSHα and TSHβ protein levels did not change significantly in other high iodine groups (Fig. 2G, H). In addition, the protein expression levels of MCT8 were highly decreased in the high iodine groups of 3-month rats, whereas no significant difference was observed in 6-month rats (Fig. 2C).

Fig. 1 The effects of subchronic and chronic high water iodine exposure on thyroid structure and function. **A** Urinary iodine levels. **B** Blood iodine levels. **C** TSH levels. **D** FT3 levels. **E** FT4 levels. **F** TPOAb levels in 3- and 6-month rats. **G** TGAb levels. **H** Body weight. **I** Thyroid weight. **J** Thyroid weight/body weight(T/B). **K** Morphological changes of thyroid pathology (×400). All data are expressed as mean ± standard deviation, $n = 10–12$ for each group. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, compared with the control group
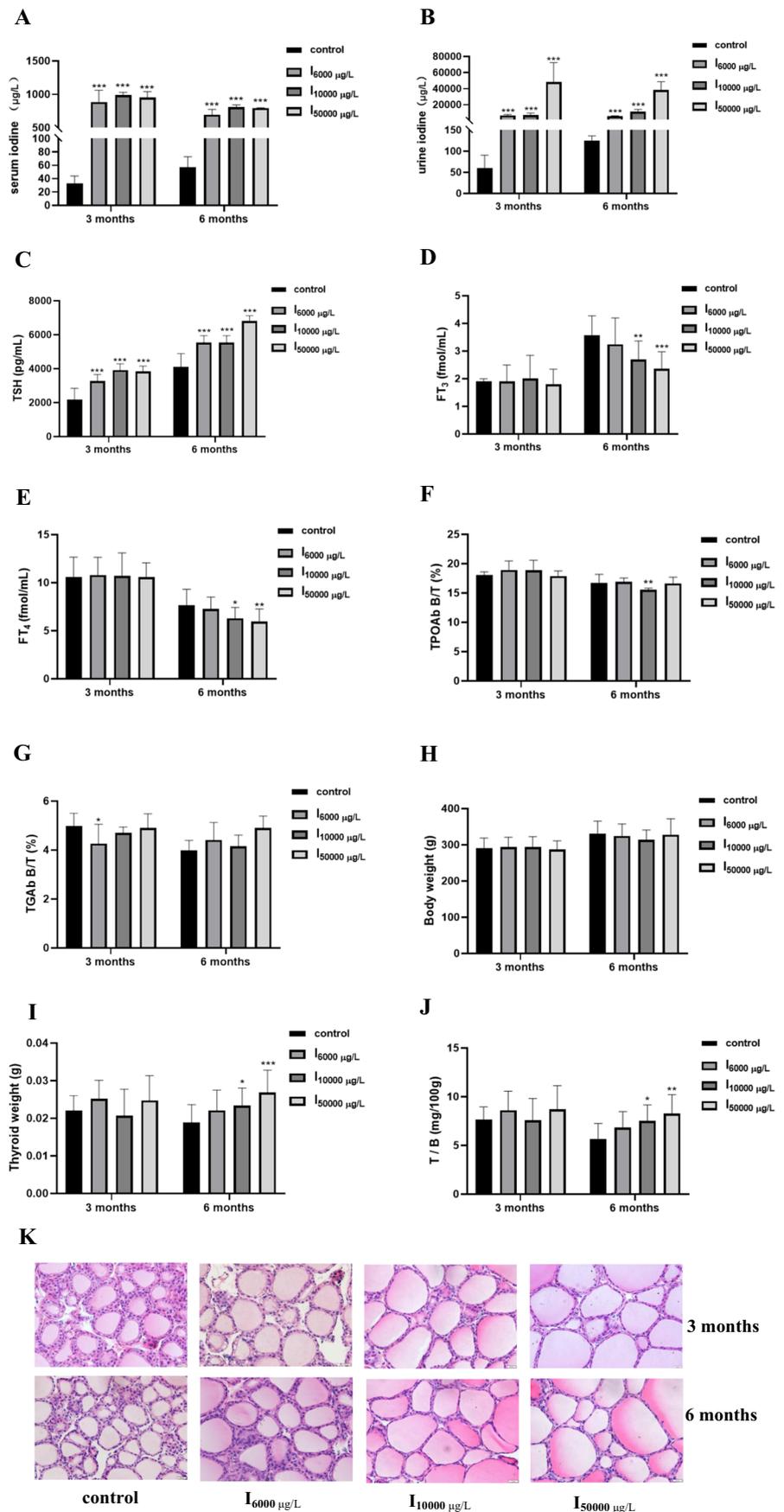
Fig. 2 The effects of iodine excess on mRNA and protein expression levels of thyroid hormone synthesis genes. **A** q-PCR detection of mRNA expression levels of NIS, Pendrin, TPO, MCT8, TSHR, TSHα, and TSHβ. **B** Representative photographs of immunohistochemistry and quantitative analysis of AOD values for NIS protein (×400). **C** Representative images of immunohistochemical photographs and quantitative analysis of AOD values of Pendrin protein (×400). **D** Representative images of immunohistochemical photographs and quantitative analysis of AOD values of TPO protein (×400). **E** Representative images of immunohistochemical photographs and quantitative analysis of AOD values of MCT8 protein (×400). (F)Representative images of immunohistochemical photographs and quantitative analysis of AOD values of TSHR protein (×400). **G** Representative images of immunohistochemical photographs and quantitative analysis of AOD values of TSHα protein (×400). **H** Representative images of immunohistochemical photographs and quantitative analysis of AOD values of TSHβ protein (×400). All data are expressed as mean ± standard deviation, $n = 6$–8 for each group.*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, compared with the control group

Fig. 2 (continued)

Pendrin (×400)

TPO (×400)

E

MCT8 (×400)

F

TSHR(×400)

Fig. 2 (continued)

G


TSHα(×400)

H

TSHβ(×400)
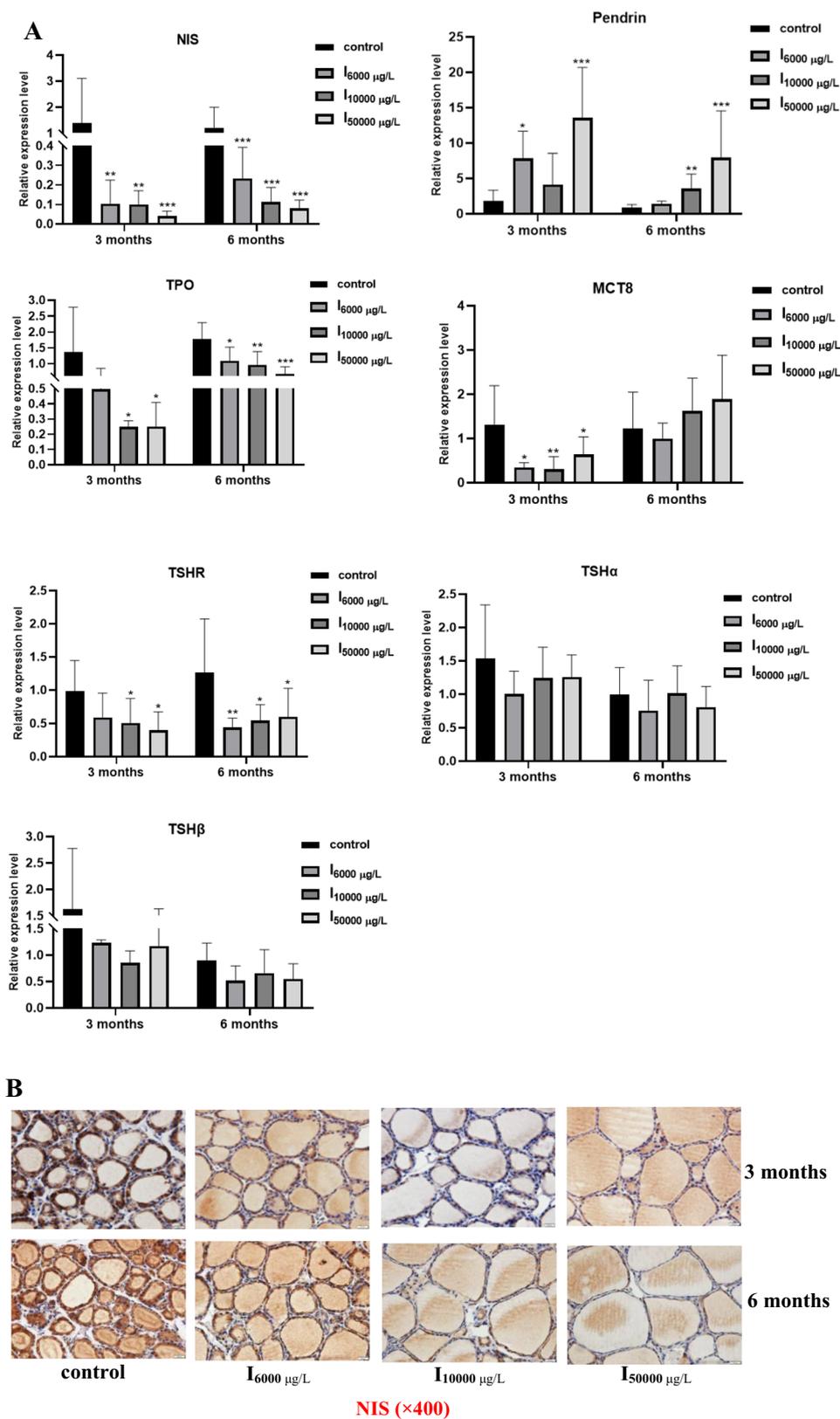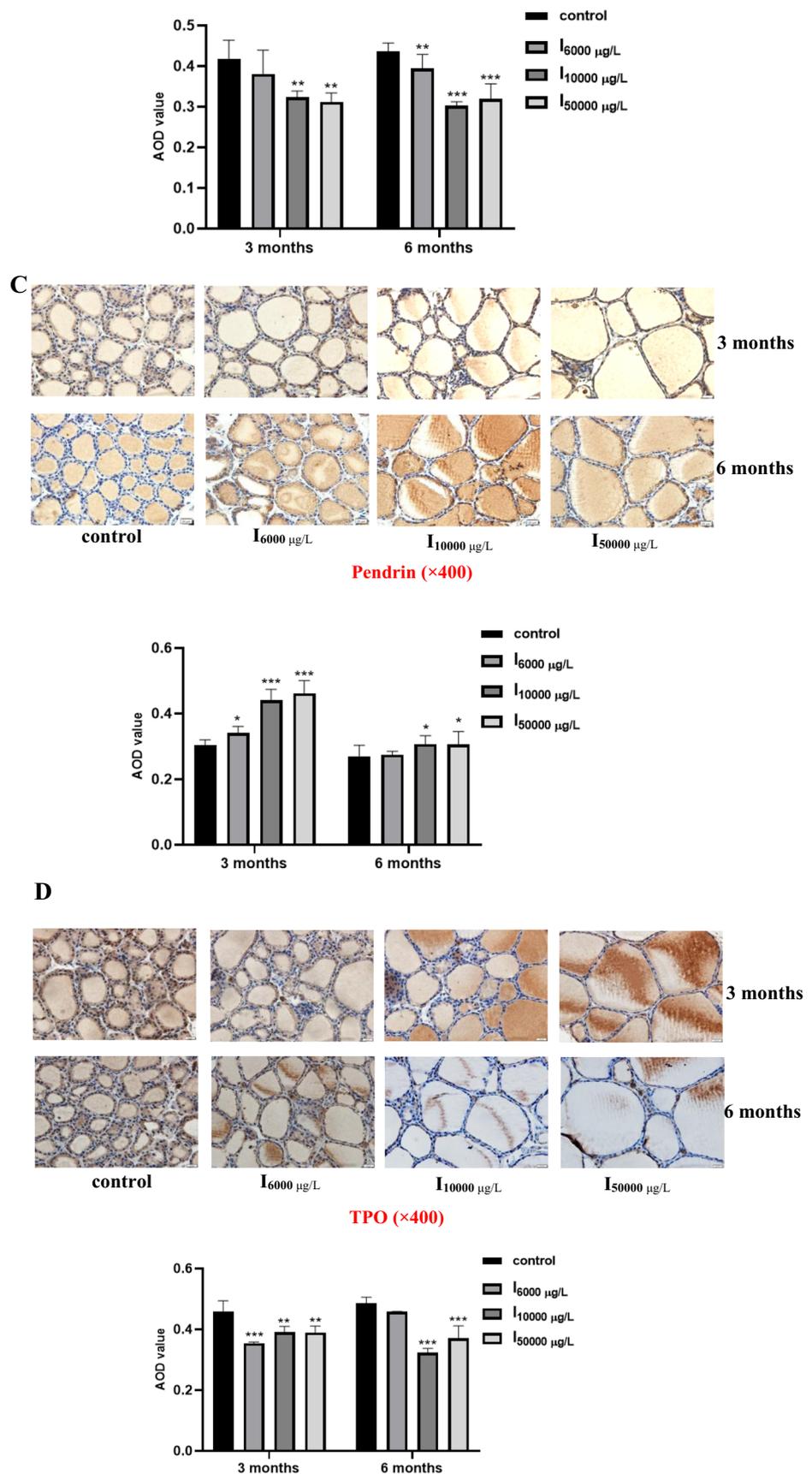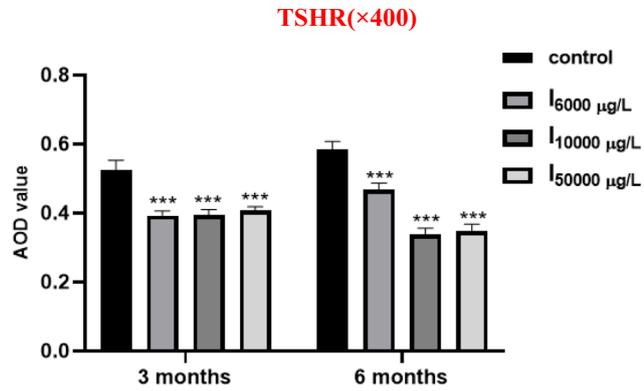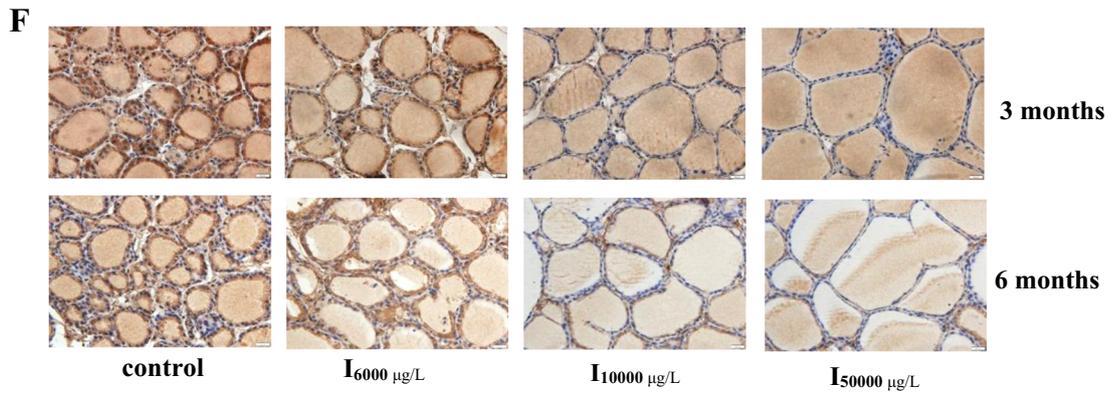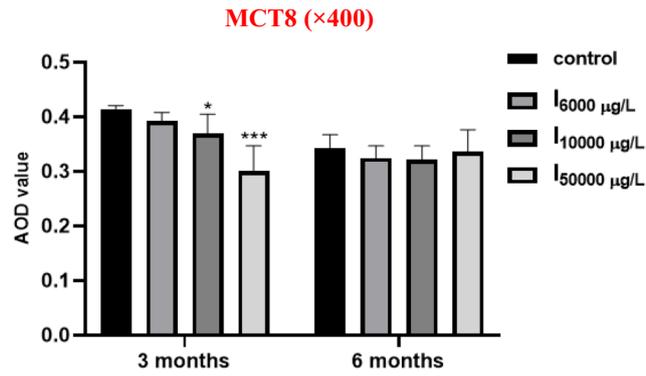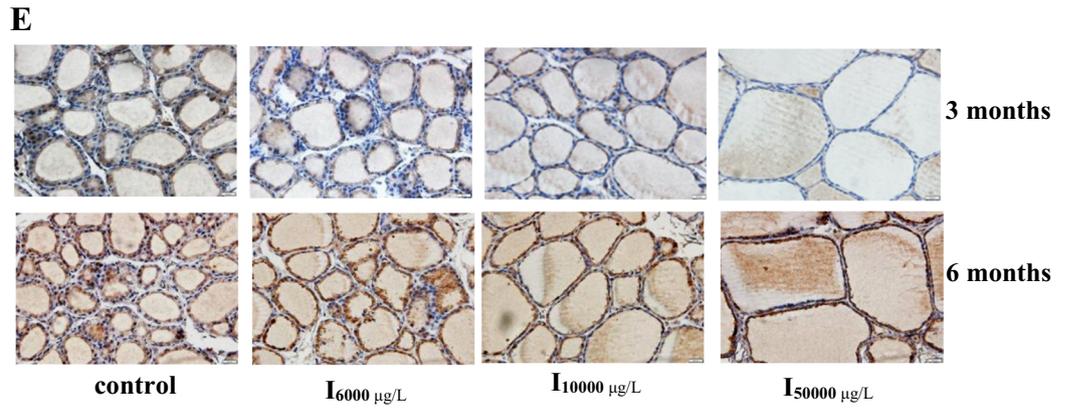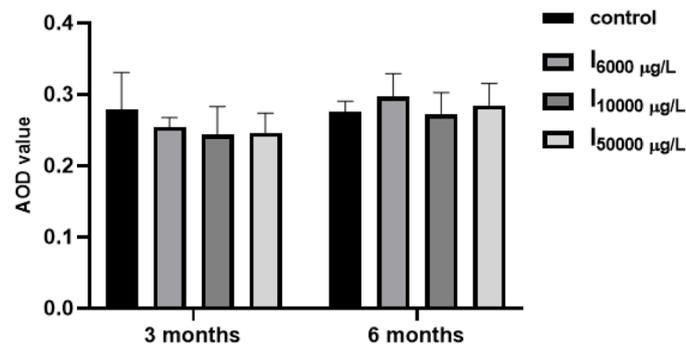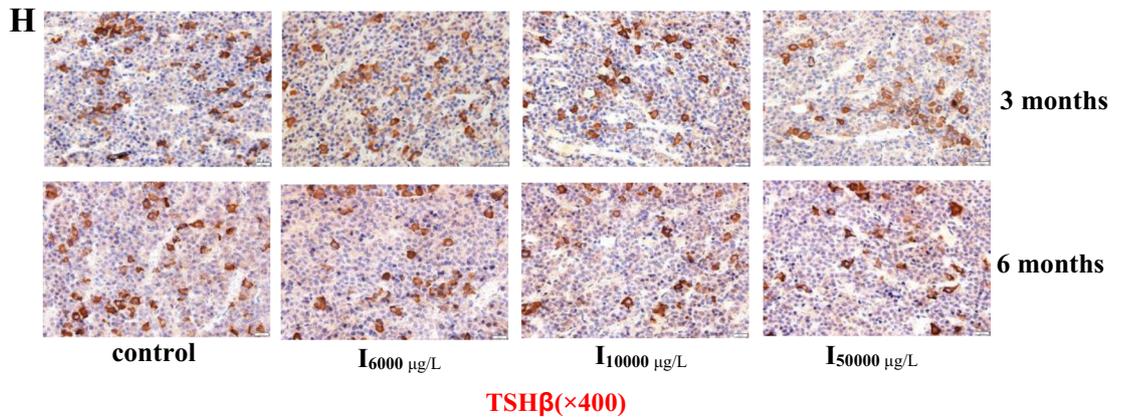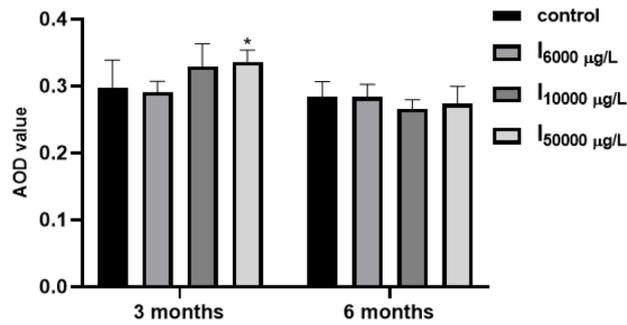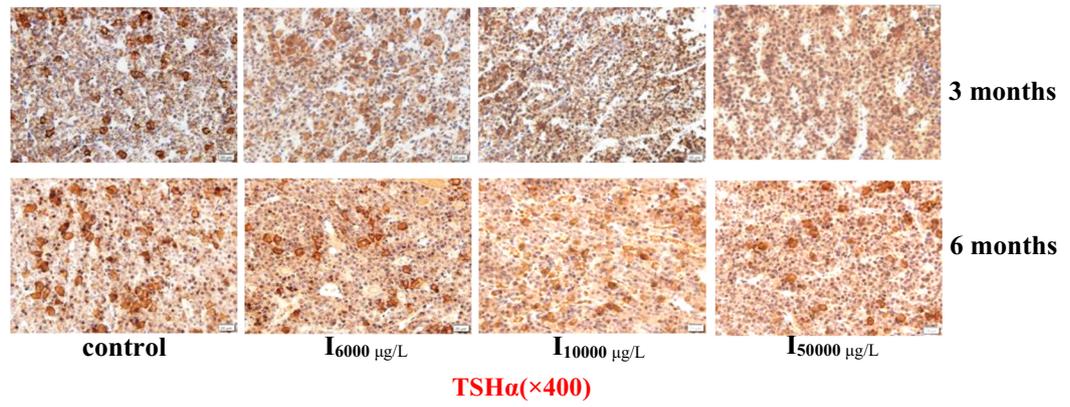
Table 2 Correlation analysis of thyroid hormone synthesis genes and TSH levels in 3 month rats

Variable	NIS		MCT8		TPO		TSHR		Pendrin	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
TSH	-0.393	**0.042**	-0.462	**0.030**	-0.493	**0.011**	-0.488	**0.010**	0.466	**0.038**

Correlation Analysis of Thyroid Hormone Synthesis Genes and Thyroid Function

The results (Table 2) showed that the mRNA levels of NIS ($r = -0.39$, $P < 0.05$), MCT8 ($r = -0.46$, $P < 0.05$), TPO ($r = -0.49$, $P < 0.05$), and TSHR ($r = -0.49$, $P < 0.05$) were negatively correlated with TSH contents under 3-month exposure, while the mRNA levels of Pendrin were positively correlated with TSH contents under 3-month exposure ($r = 0.47$, $P < 0.05$). For 6 months' exposure, the results (Table 3) showed that NIS ($r = -0.79$, $P < 0.05$), TPO ($r = -0.46$, $P < 0.05$), and TSHR ($r = -0.47$, $P < 0.05$) mRNA levels were negatively correlated with TSH content, NIS ($r = 0.46$, $P < 0.05$), TPO ($r = 0.57$, $P < 0.05$), and TSHR ($r = 0.55$, $P < 0.05$) mRNA levels were correlated with FT3, NIS ($r = 0.50$, $P < 0.05$), TPO ($r = 0.48$, $P < 0.05$), and TSHR ($r = 0.58$, $P < 0.05$) mRNA levels were positively correlated with FT4 contents. Moreover, Pendrin mRNA levels were positively correlated with TSH contents ($r = 0.44$, $P < 0.05$) and negatively correlated with FT3 ($r = -0.61$, $P < 0.05$) and FT4 ($r = -0.53$, $P < 0.05$) contents.

MiRNAs Profiling Related with Thyroid Hormone Synthesis Genes

To further explore the miRNAs profiling related with the synthesis of thyroid hormones, we predicted miRNAs for NIS, Pendrin, TPO, MCT8, and TSHR, using TargetScan, miRWalk, and MiRDB databases, and miRNAs those regulate more thyroid hormone synthesis genes were preferentially chosen. The results showed that miR-200b-3p, miR-675-5p, miR-883-5p targeted NIS; miR-200b-3p, miR-185-5p, and miR-24-3p targeted MCT8; miR-185-5p, miR-24-3p, miR-200a-3p, miR-25-3p- and miR-883-5p targeted TSHR, miR-300-3p, miR-200a-3p, and miR-25-3p targeted TPO; and miR-1839-3p targeted Pendrin and the target binding sites were shown in Fig. 3 A–E.

The q-PCR results showed that compared with the control group, levels of miR-200b-3p, miR-185-5p, miR-24-3p, miR-200a-3p, and miR-25-3p in the high iodine groups were significantly increased under 3 months' exposure (Fig. 3F). However, the levels of miR-675-5p, miR-883-5p, and miR-300-3p were not changed significantly (Fig. S1B), and miR-1839-3p levels were significantly decreased in the high-iodine groups exposed to high iodine for 3 or 6 months (Fig. 3F, G). Nevertheless, there was no significant change in levels of miR-200b-3p, miR-185-5p, miR-24-3p, miR-200a-3p, and miR-25-3p in the high iodine groups after 6 months' exposure (Fig. S1A), while the levels of miR-675-5p and miR-883-5p were significantly increased in I$_{10000 \mu g/L}$ and I$_{50000 \mu g/L}$ groups, and miR-300-3p levels were significantly higher in all high iodine groups (Fig. 3G).

Correlation Analysis to Verify the Relationship of mRNAs with Related miRNAs

The correlation analysis of NIS, Pendrin, TPO, MCT8, and TSHR mRNA levels with related miRNAs was performed, and the results showed (Table 4) that miR-200b-3p was negatively correlated with NIS mRNA ($r = -0.68$, $P < 0.05$), miR-200b-3p ($r = -0.60$, $P < 0.05$), miR-185-5p ($r = -0.58$, $P < 0.05$), and miR-24-3p ($r = -0.56$, $P < 0.05$) were negatively correlated with MCT8 mRNA; miR-185-5p ($r = -0.64$, $P < 0.05$), miR-24-3p ($r = -0.59$, $P < 0.05$), miR-200a-3p ($r = -0.59$, $P < 0.05$), and miR-25-3p ($r = -0.62$, $P < 0.05$) were negatively correlated with TSHR mRNA; miR-200a-3p ($r = -0.53$, $P < 0.05$) and miR-25-3p ($r = -0.75$, $P < 0.05$) were negatively correlated with TPO mRNA; and miR-1839-3p was negatively correlated with Pendrin mRNA ($r = -0.59$, $P < 0.05$) in rats after 3 months' exposure. For 6 months' rats (Table 5), miR-675-5p ($r = -0.53$, $P < 0.05$) and miR-883-5p ($r = -0.60$, $P < 0.05$) were negatively correlated with NIS mRNA, miR-883-5p were negatively correlated with TSHR mRNA ($r = -0.50$,

Table 3 Correlation analysis of thyroid hormone synthesis genes and thyroid function in 6 month rats

Variable	NIS		TPO		TSHR		Pendrin	
	r	*P*	r	*P*	r	*P*	r	*P*
TSH	-0.794	**0.000**	-0.464	**0.017**	-0.468	**0.043**	0.435	**0.043**
FT3	0.459	**0.028**	0.572	**0.008**	0.545	**0.038**	-0.606	**0.008**
FT4	0.501	**0.018**	0.479	**0.038**	0.582	**0.040**	-0.527	**0.032**

Fig. 3 Prediction and expression of miRNAs targeting thyroid hormone synthesis genes. **A** Predicted binding sites of rno-miR-200b-3p, rno-miR-675-5p, and rno-miR-883-5p to slc5a5 (NIS). **B** Predicted binding sites of rno-miR-200b-3p, rno-miR-185-5p, and rno-miR-24-3p to slc16a2 (MCT8). **C** Predicted binding sites of rno-miR-1839-3p to slc26a4 (Pendrin). **D** Predicted binding sites of rno-miR-185-5p, rno-miR-24-3p, rno-miR-200a-3p, rno-miR-25-3p, and rno-miR-883-5p to TSHR. **E** Predicted binding sites of rno-miR-200a-3p, rno-miR-25-3p, and rno-miR-300-3p to TPO. **F** Expression levels of miR-200b-3p, miR-24-3p, miR-185-5p, miR-200a-3p, miR-25-3p, and miR-1839-3p in 3-month rats. **G** Expression levels of miR-675-5p, miR-883-5p, miR-300-3p, and miR-1839-3p in 6-month rats. All data are expressed as mean ± standard deviation, $n = 6$–8 for each group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, compared with the control group
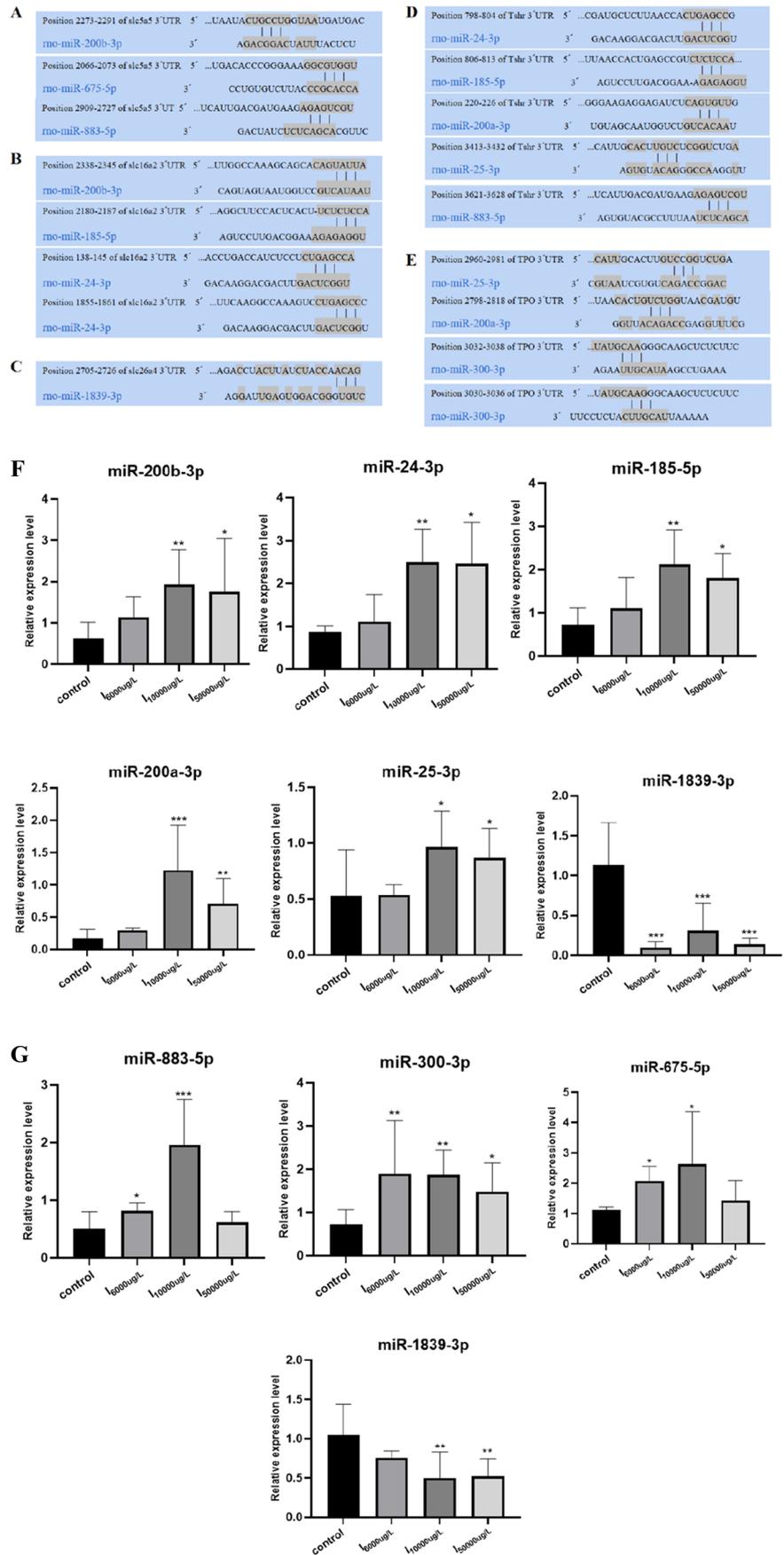
Table 4 Correlation analysis of thyroid hormone synthesis genes and related miRNA in 3 months rats

Variable	miR-200b-3p		miR-185-5p		miR-24-3p		miR-200a-3p		miR-25-3p		miR-1839-3p	
	r	P	r	P	r	P	r	P	r	P	r	P
NIS	-0.676	**0.014**	_	_	_	_	_	_	_	_	_	_
MCT8	-0.599	**0.034**	-0.582	**0.025**	-0.560	**0.049**	_	_	_	_	_	_
TPO	_	_	_	_	_	_	-0.532	**0.044**	-0.749	**0.003**	_	_
TSHR	_	_	-0.642	**0.007**	-0.588	**0.038**	-0.593	**0.036**	-0.618	**0.021**	_	_
Pendrin	_	_	_	_	_	_	_	_	_	_	-0.594	**0.017**

$P < 0.05$), miR-300-3p were negatively correlated with TPO mRNA ($r = -0.46$, $P < 0.05$), and miR-1839-3p was negatively correlated with Pendrin mRNA ($r = -0.44$, $P < 0.05$).

Discussion

In this study, we established subchronic and chronic high iodine exposure rat model. The subclinical hypothyroidism and goiter occurred in rats exposed to high iodine for three months, while hypothyroidism and more serious goiter occurred in 6 months' exposure. In addition, from the thyroid-pituitary axis, the influence of subchronic to chronic high iodine exposure on thyroid hormone synthesis genes was comprehensively studied. The results showed that NIS, Pendrin, TPO, MCT8, and TSHR were remarkably altered in the high iodine groups and significantly correlated with TSH contents. Then, we predicted that miRNAs may play a role by regulating thyroid hormone synthesis genes, and the results showed that the expression levels of some miRNAs changed significantly under high iodine exposure, and suggested that negative correlation with their related targeted genes.

The national survey, carried out in 2017, found that the iodine content in drinking water in Chengbu County, Shaoyang City, Hunan Province, is as high as 15,000 µg/L, which is more than 150 times of the normal iodine demand of human body [18]. Considering that rats have a stronger tolerance to high iodine, which is about 25–50 times of human beings, thus the highest dose of iodine is 50,000 µg/L in this study. The intermediate dose of 10,000 µg/L was chosen according to the dose–response relationship. In addition, our previous study found obvious hypothyroidism and goiter in rats exposed to 6000 µg/L KIO_3, thus the minimum excess

exposure dose of iodine was set at 6000 µg/L in this study. Li et al. found that Wistar rats given 10HI(1245 µg/L) and 50HI (6778 µg/L) developed subclinical hypothyroidism at 8–24 weeks [19]; our group previously found that Wistar rats exposed to 3000 µg/L KIO_3 for 3 months developed subclinical hypothyroidism and those developed hypothyroidism exposed to 6000 µg/L KIO_3 for 3 months [20]; consistent with the previous studies, the present study found subclinical hypothyroidism occurred in rats exposed for three months, and hypothyroidism after 6 months. In addition, H&E staining showed that compared to the control group, the thyroid follicular epithelial cells in the high iodine group were flat and the follicular lumen increased in size, and became more severe with the increasing iodine dose.

In recent years, as is known to us, only Manna et al. focused on the process of subchronic to chronic high iodine exposure, and they found a significant decrease in TPO activity and NIS expression in Wistar rats consuming 4, 6, 12, and 24 µg of iodine per day for 1, 2, 4, and 8 months [21], while other genes were not studied. TSH is the main positive regulator of TPO expression in thyroid cells, thus it is thought that decreased TPO expression may be due to impaired TSH signaling cascades. TSHR, as a receptor for TSH, plays an important regulatory role in thyroid hormone synthesis. Extensive studies have shown that high iodine exposure significantly reduces the activity and expression of NIS, TPO and TSHR. Similarly, we found decreased expression levels of TPO and TSHR in this study.

It has been suggested that TSH positively regulates the expression of Pendrin in rat thyroid cells through a post-transcriptional mechanism [22]; Calil-Silveira J et al. found that intracellular iodine induced increased expression of Pendrin when iodine excess was present [23]; however,

Table 5 Correlation analysis of thyroid hormone synthesis genes and related miRNA in 6 month rats

| Variable	miR-675-5p		miR-883-5p		miR-300-3p		miR-1839-3p	
	r	P	r	P	r	P	r	P
NIS	-0.532	**0.036**	-0.596	**0.021**	_	_	_	_
TPO	_	_	_	_	-0.459	**0.042**	_	_
TSHR	_	_	-0.444	**0.023**	_	_	_	_
Pendrin	_	_	_	_	_	_	-0.436	**0.029**

Fig. 4 Regulation of related miRNAs on NIS, Pendrin, TPO, MCT8, and TSHR during thyroid hormone synthesis after subchronic high iodine exposure
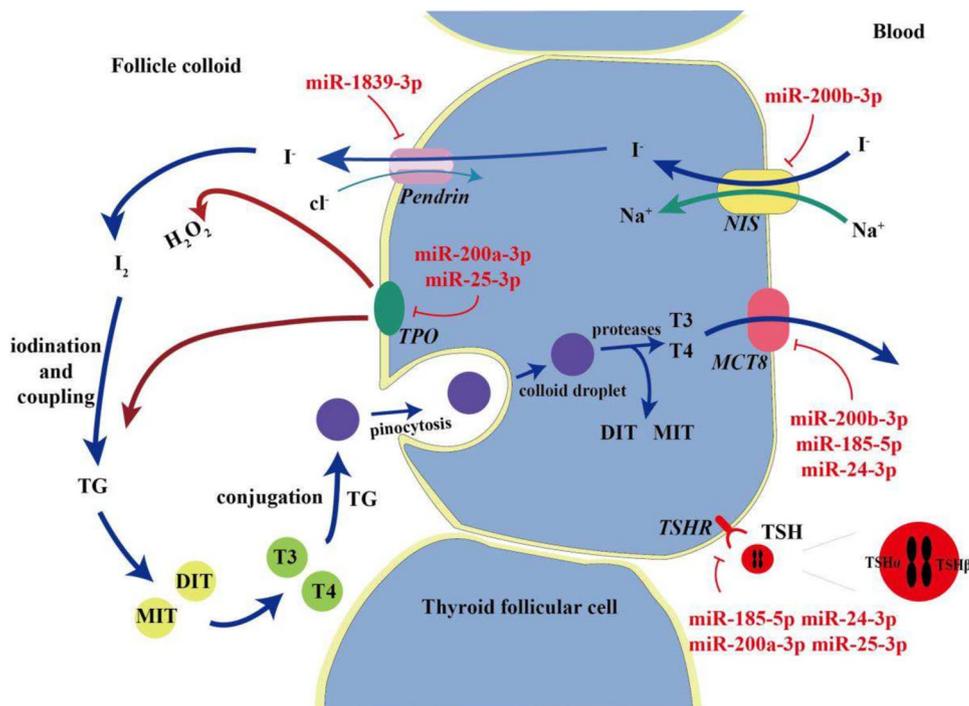
the exact physiological role of Pendrin in mediating iodine efflux is still controversial. Our results showed a significant increase in the expression level of Pendrin. Monocarboxylate transporter 8 (MCT8) is a transporter protein located in the basolateral membrane, is involved in T3 and T4 transport. Di Cosmo C et al. demonstrated for the first time that MCT8 can be involved in thyroid hormone secretion by

administering TSH to MCT8 knockout mice [24]; In addition, MCT8 has role in the Wolff-Chaikoff and escape effects under high iodine exposure [6]. In this study, subchronic high iodine resulted in a significant decrease in MCT8 mRNA and protein levels, whereas no significant effect was observed under the chronic high iodine exposure. Ningna Li et al. also found no significant effect on MCT8 protein and

Fig. 5 Regulation of related miRNAs on NIS, Pendrin, TPO, and TSHR during thyroid hormone synthesis after chronic high iodine exposure
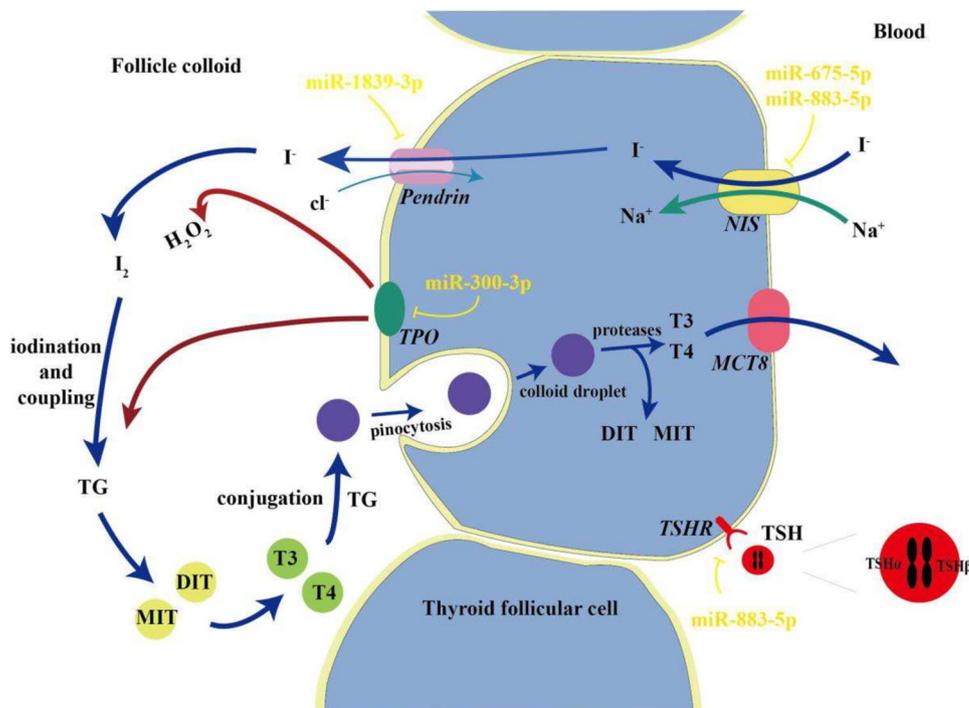
mRNA levels after 24 weeks of exposure in rats given 3HI (277 µg/L), 6HI (697 µg/L), 10HI (1245 µg/L), and 50HI (6778 µg/L) [19]. We suggested that subchronic high iodine exposure significantly reduces the expression of MCT8 in thyroid tissue probably due to the lysosomal degradation of MCT8 protein, and extended high iodine exposure leads to a weakening or loss of the inhibitory effect of the MCT8 gene, thus restoring the synthesis of thyroid hormones in the body as much as possible.

Studies have found that increased TSHα and TSHβ mRNA expression, and decreased TSHα and TSHβ protein levels in the pituitary of female rats treated with excess iodine [25]; Calil-Silveira J et al. found no significant change in both protein and mRNA expression of TSHα under high iodine exposure and only 0.05% mRNA levels of TSHβ in the HI group increased[5], which is similar to the results of the present study. We found that the level of TSHα protein was significantly increased in the $I_{50,000 \, \mu g/L}$ group after 3 months' exposure, while TSHα mRNA level was not significantly altered. Considering that miRNA plays a role by regulating the degradation of mRNA, thus we did not detect the levels of related miRNAs. However, the mechanism of TSHα protein increase exposed to $I_{50,000 \, \mu g/L}$ need to be further explored. In addition, levels of TSHα and TSHβ mRNA and protein in other groups were not changed significantly, suggesting that high iodine exposure may mainly increase serum TSH levels.

Accumulating studies have found that miRNAs are involved in various diseases, such as cancer, inflammation and metabolic diseases. Our results showed that NIS, Pendrin, TPO, MCT8, and TSHR were significantly altered under three and 6 months' exposure, respectively. The results of database screening showed that miR-200b-3p, miR-675-5p, and miR-883-5p could target the 3'UTR region of NIS gene. Current studies on miRNA regulation of NIS are limited to thyroid cancer tissues and cells, such as miR-181a-5p, miR-146b-3p, and miR-206 in papillary thyroid cancer [26–28]; miR-875-5p in hypofractionated thyroid cancer [29] and let-7f-5p in follicular thyroid cancer [30] can regulate the expression of NIS. However, studies on miR-200b-3p and miR-675-5p found aberrant regulation in various cancers, such as nasopharyngeal, colorectal, pancreatic, lung, and breast cancer, that miR-675-5p can directly target MAPK1 to inhibit the oncogenicity of papillary thyroid carcinoma, while no study reported on miR-883-5p. In this study, we found that NIS plays an important role in CH (Hypothyroidism) and SCH (subclinical hypothyroidism) caused by high iodine, miR-200b-3p, miR-675-5p, and miR-883-5p may affect the function and structure of the thyroid by targeting and regulating the expression of NIS. Similarly, no studies on miRNAs regulating Pendrin. The present study showed that level of miR-1839-3p significantly decreased in 3 and 6 months' high iodine exposure rats, the expression level of Pendrin mRNA increased significantly, and the correlation analysis suggested a possible regulation.

MiR-300-3p, miR-200a-3p, and miR-25-3p were found to target TPO through the database screening. A previous study found that miR-200a-5p was negatively correlated with TPO in PTC [31]. MiR-200a-3p and miR-200a-5p are produced by two branches of the same precursor processed separately, some studies have demonstrated that miR-200a-3p and miR-200a-5p have synergistic and co-regulatory effects [32]. Therefore, we suggested that miR-200a-3p may also function by regulating TPO. MiR-300-3p and miR-25-3p were found to play a role in various diseases, such as induction of vascular permeability and angiogenesis [33], protecting chondrocytes [34], regulating adipocyte differentiation [35], whereas some studies found miR-300-3p act as a potential marker for transient ischemic attack [36]. The role of miR-300-3p and miR-25-3p in thyroid disease has not been reported yet. The present study showed that elevated expression levels of miR-300-3p in 6-month high iodine exposure rats, and miR-200a-3p and miR-25-3p in 3-month high iodine exposure rats had a significant negative correlation with TPO, suggesting that miR-300-3p, miR-200a-3p, and miR-25-3p may have a target-regulatory relationship with TPO, thus affecting the thyroid function.

Database screening showed that miR-200b-3p, miR-185-5p, and miR-24-3p can target MCT8. MiR-375 was found to regulate MCT8 expression in medullary thyroid carcinoma [37]. MiR-185-5p was found to be lowly expressed in follicular thyroid cancer [38]. MiR-24-3p is currently used as a therapeutic target in a variety of cancers, decreased miR-24-3p expression inhibits the cell growth of PTC [39]. MiR-200b-3p, miR-184-5p, and miR-24-3p in SCH and CH have not been reported yet. In the present study, the expression levels of miR-200b-3p, miR-185-5p, and miR-24-3p were significantly elevated under the high iodine exposure, and the correlation analysis showed that they all were negatively correlated with MCT8, suggesting that miR-200b-3p, miR-185-5p, and miR-24-3p may target MCT8 in the progression of SCH and CH. At present, there is no literature reporting that miRNAs can regulate the expression of TSHR in thyroid tissue. Through database prediction, we found that miR-185-5p, miR-24-3p, miR-200a-3p, miR-25-3p, and miR-883-5p can bind to TSHR and are highly expressed in thyroid tissue, and these miRNAs have not been found to be involved in the progression of SCH and CH.

The present study is the first to explore the changes in the expression levels of a cluster of thyroid hormone synthesis genes from subchronic to chronic high iodine exposure, and the role of related miRNAs. This study comprehensively explored the effects of thyroid hormone synthesis genes in the pituitary-thyroid axis in response to high water iodine exposure, and the changes in the progression from subclinical hypothyroidism to hypothyroidism.

More studies on miRNAs regulating thyroid hormone synthesis genes have focused on thyroid cancer, while fewer studies have been conducted on other thyroid diseases, such as hypothyroidism. Our results showed that the structural and functional shift of the rat thyroid from SCH to CH resulted in altered expression levels of miRNAs profiling, suggesting that miRNAs play different roles in different pathological status. However, there are a few limitations in this study. Firstly, the targeting relationship was not further verified by gene knockdown experiments, cell transfection and etc. Secondly, it is currently believed that lncRNA and circRNA can affect the expression level of coding genes by regulating miRNAs [40], so we will further explore the effects of lncRNA and circRNA on gene expression.

Taken together, the miRNA expression profile of genes regulating thyroid hormone synthesis is significantly altered from subclinical hypothyroidism to hypothyroidism induced by high iodine exposure, and some miRNAs may play an important role in SCH (Fig. 4) or CH (Fig. 5) through regulating NIS, Pendrin, TPO, MCT8, and TSHR, providing promising targets to alleviate the pathological changes of thyroid structure and function.

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Declarations

Competing Interests The authors declare no competing interests.

Ethics Approval The experimental protocol was approved by the Animal Ethics Committee of Harbin Medical University (hrbmu-ecdc20180302).

Conflict of Interest The authors declare no competing interests.

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Authors and Affiliations

Chunyu Zhang[1] · Jinyin Yao[2] · Chang Liu[1] · Kunying Yang[1] · Wei Zhang[1] · Dianjun Sun[1] · Weikuan Gu[3]

✉ Wei Zhang
zwhxd@126.com

✉ Dianjun Sun
hrbmusdj@163.com

✉ Weikuan Gu
wgu@uthsc.edu

Chunyu Zhang
zcy17853735037@163.com

Jinyin Yao
sdslyyjy@163.com

Chang Liu
liuc_hmu@163.com

Kunying Yang
yangky1209@163.com

[1] Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, Key Lab of Etiology and Epidemiology, Education Bureau of Heilongjiang Province &, Heilongjiang Provincial Key Laboratory of Trace Elements and Human Health, Ministry of Health, 157# Baojian Road Harbin, 150081 Harbin, China

[2] Department of Public Health, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong, China

[3] Department of Orthopedic Surgery and BME-Campbell Clinic, University of Tennessee Health Science Center, Memphis, TN 38163, USA