Endocrine Journal 2017, **64** (4), 437-447

ORIGINAL

Natural history of mild subclinical hypothyroidism in a middle-aged and elderly Chinese population: a prospective study

Xiang Li[1)], Donghu Zhen[2)], Meng Zhao[1)], Lu Liu[1)], Qingbo Guan[1)], Haiqing Zhang[1)], Shujian Ge[3)], Xulei Tang[2)] and Ling Gao[4)]

[1)] *Department of Endocrinology and Metabolism, Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong 250021, China*
[2)] *Department of Endocrinology and Metabolism, The First Hospital of Lanzhou University, Lanzhou 730000, China*
[3)] *Department of Science and Education, Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong 250021, China*
[4)] *Scientific Center, Shandong Provincial Hospital affiliated to Shandong University, Shandong 250021, China*

Abstract. Subclinical hypothyroidism (SCH) has a high global prevalence. Most SCH patients have mild cases (thyrotropin ≤10 mIU/L). Treatment recommendations for mild SCH are controversial, which raises concerns about the natural history of mild SCH. We aimed to clarify the natural history of mild SCH. This is a prospective population-based study. We measured thyroid function in 11,000 participants in the REACTION study and followed 505 newly diagnosed mild SCH patients aged 40-years or older between 2011 and 2014. Logistic regression analysis was used to seek baseline parameters associated with the natural outcomes of mild SCH. Among 505 mild SCH patients, 221 (43.8%) had persistent SCH, 251 (49.7%) reverted to euthyroidism, and 17 (3.4%) progressed to overt hypothyroidism (OH). Patients with higher baseline total cholesterol (TC, between 201.0-240.0 mg/dL or >240.0 mg/dL *vs*. <201.0 mg/dL, $p = 0.048$ and 0.006, respectively) or positive thyroid peroxidase antibodies (TPOAb, $p = 0.009$) had higher risks of progression to OH, while those with higher baseline creatinine (CR, between 0.71-0.80 mg/dL or >0.80 mg/dL *vs*. ≤0.65 mg/dL, $p = 0.031$ and 0.004, respectively), higher baseline thyrotropin (≥7 mIU/L, $p < 0.001$) or older (>60 years *vs*. ≤50 years, $p = 0.012$) had lower odds of reverting to euthyroidism. In conclusion, TPOAb and TC seem to be more important predictors of progression to OH than initial thyrotropin, whereas high baseline thyrotropin or CR were negative correlated with reversion to euthyroidism. The prognostic value of TC and CR in mild SCH should be considered.

Key words: Natural history, Hypothyroidism, Thyrotropin, Prospective study, Risk factors

SUBCLINICAL HYPOTHYROIDISM (SCH), characterized as a state of elevated thyrotropin (TSH) with normal levels of serum free thyroxine (FT4), can be mild (TSH ≤10 mIU/L) or severe (TSH >10 mIU/L). This has become a common thyroid disease among adults [1-4]. The prevalence of SCH varies between 4% and 20% in the adult population [4]. Age, sex, race, body mass index (BMI), iodine intake and cut-off levels used to define SCH among different studied populations all contribute to the wide range of prevalence [3-5].

The natural history of SCH also fluctuated among several population-based studies. The risk of progression to overt hypothyroidism (OH) ranged between 2% and 28%. On the other hand, the proportion of patients with spontaneous normalization of thyroid function is 20%-53.5%. Age, sex, initial TSH levels and thyroid peroxidase antibodies (TPOAb) status may influence thyroid function [4, 6-12]. However, when referring to the natural history of "mild" SCH, only Rosário *et al*. have focused on this topic in only the female population, and found the prognostic value of ultrasound [7, 9].

The clinical significance of SCH varies widely. A recent meta-analysis found that SCH is related to an increased risk of coronary heart disease events and

Submitted Nov. 14, 2016; Accepted Jan. 10, 2017 as EJ16-0549
Released online in J-STAGE as advance publication Mar. 7, 2017
Correspondence to: Xulei Tang, Ph.D., Department of Endocrinology and Metabolism, The First Hospital of Lanzhou University, No.1, West Donggang Road, Lanzhou 730000, China.
E-mail: xulei_tang@126.com
Correspondence to: Ling Gao, Ph.D., Scientific Center, Shandong Provincial Hospital affiliated to Shandong University, No.324, Jing 5 Road, Jinan, Shandong 250021, China.
E-mail: gaoling1@medmail.com.cn

mortality in those with higher TSH levels [13]. Other possible adverse consequences include depression, Alzheimer disease, the worsening of renal function in patients with chronic kidney diseases (CKD), symptomatic OH and disordered lipid metabolism, particularly elevation of total cholesterol (TC) [2, 4]. It is widely agreed that L-thyroxine is necessary for severe SCH patients. However, controversy exists for mild SCH patients [1, 2, 4].

The predictive value of TSH levels, TPOAb status and ultrasound for the natural history of the disease have been investigated [6-12]. However, important metabolic indices such as TC or creatinine (CR) have not been researched. Due to the essential role of the natural history of mild SCH in treatment decision-making and the lack of knowledge about it, we performed this prospective population-based cohort study to investigate the natural history of mild SCH in a Chinese population aged 40-years or older, for whom the spontaneous course had not ever been studied. We focused on factors associated with the progression or recovery of thyroid function, and we expected to find innovative prognostic factors.

Materials and Methods

Subjects

This was a prospective population-based cohort study conducted in the rural area of Shandong Province, China. The study population was derived from the REACTION study, which was a community-based study designed to investigate the epidemiology of metabolic diseases across China since 2011 [14]. Inhabitants aged 40-year or older who had lived in their residences for over 5 years were invited to participate. Nearly 11,000 individuals were enrolled in the original cohort. We performed the first visit from Apr 1, 2011 to Jan 31, 2012, and the final visit from Jul 1, 2014 to Jan 31, 2015.

We used following exclusion criteria: 1) missing demographic data such as BMI; 2) a history of diagnosed thyroid diseases or thyroid disease treatment including thyroidectomy, radioiodine therapy, anti-thyroid medication or thyroid hormone; 3) taking medicine that influences thyroid function, renal function or lipid metabolism, including amiodarone, lithium, tyrosine kinase inhibitors, alemtuzumab, interferon, angiotensin-converting enzyme inhibitors, statins and fibrates, as well as medicines that influence the process of thyroid function analysis, including glucocorticoids, estrogens, non-steroid anti-inflammatory drugs, antiepileptic drugs, rifampin, furosemide or heparin, in the last 3 months [15]; or 4) pregnancy during the cohort. We contacted the eligible subjects by telephone every 2 months and conducted physical examination three times during the 3-year follow-up.

The study was approved by the Ethics Committee of Shanghai Jiao Tong University and all participants signed informed consent [14].

Anthropometric measurements and laboratory methods

All investigators were trained to obtain anthropometric data in line with a standard protocol to minimized the measurement error. Height and weight were measured in centimeters (cm) and kilograms (kg), respectively. BMI was calculated by dividing weight (kg) by squared height (m^2). Blood pressure was measured three times in the sitting position after a 5 minutes' rest for each participant using an electronic sphygmomanometer and the average of three times measurements was calculated for further study. Every participant was inquired to complete a standard questionnaire, and we didn't instruct them change their dietary habits.

All participants provided blood samples from the antecubital vein between 0800 h and 1000 h after a minimum 10 hours fast. An oral glucose tolerance test was performed if the participants had no previous diagnosis of diabetes. Subjects were asked to ingest a solution containing 75g glucose, and venous blood samples were collected at 120 min for evaluating plasma glucose levels [16]. Serum levels of blood glucose were measured directly within 2 hours. Blood samples were centrifuged, subpackaged, and shipped on dry ice. Thyroid function, including TSH, free triiodothyronine (FT3), FT4 and TPOAb levels, was measured using chemiluminescent procedures (Cobas E601; Roche, Basel, Switzerland). The ARCHITECT ci16200 Integrated System (Abbott) were used to determine the serum lipids profile, liver function and renal function. The intra-assay and inter-assay coefficients of variation were below 5% for all parameters.

The laboratory reference range were as follows: FT3, 2.01-4.42 pg/mL (3.1-6.8 pmol/L); FT4, 0.94-1.72 ng/dL (12-22 pmol/L); TSH, 0.27-4.2 mIU/L. SCH was defined as a combination of elevated TSH and normal FT4, and classified into mild or severe when TSH $\leq$10 mIU/L or >10 mIU/L, respectively [4]. Positivity for TPOAb was considered when the serum

concentration of this antibody was higher than 34 IU/mL. Diabetes was diagnosed when the subjects met as least one criteria follows: a self-reported previous diagnosis by health care professionals, fasting plasma glucose level of 126 mg/dL (7.0 mmol/L) or higher, 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher, or HbA1c concentrations of 6.5% or more [17]. Hypertension was defined as a history of taking blood pressure lowing agents in last 3 months or a blood pressure of 140/90 mm Hg (18.7/12.0 kPa) or greater [18]. Menopause is defined as 1 year without menses [19].

Statistical analyses

Results are shown as the mean ± SD, median (interquartile range) or number (percentage). Differences among groups were compared using the one-way ANOVA test, the χ^2 test or the Kruskal-Wallis test. If the null hypothesis was rejected in any test, we used Fisher's least significant difference test or the Dunnett-t3 test for ANOVA testing, Bonferroni correction for χ^2 testing and Kruskal-Wallis test to perform multiple comparisons. To analyze the variables related to the transition from SCH to OH or euthyroidism, logistic regression models were used. Aimed to find significant variables, we substituted all factors that might influence the natural history of SCH into the regression equations simultaneously without the effect of obvious collinearity. In order to associate the results with clinical significance and so further develop the therapeutic strategy, each parameter was stratified according to clinical consensus, except for CR and gamma-glutamyl transpeptidase (GGT), which were classified according to quartiles, because the values of CR and GGT of most subjects were within the normal range.

All calculated p values were two-sided, and differences were considered significant if p <0.05 except for the p value of multiple comparisons, which was determined by specific methods. All statistical analyses were performed using SPSS Statistical Analysis System (version 23.0 for Windows; SPSS Inc, Chicago, IL, USA).

Results

To eliminate the concern that the 90 eligible subjects who did not accomplished the cohort might influence our overall results, we compared baseline characteristics between 505 subjects who accomplished cohort and those did not and found that subjects who accomplished cohort had higher high-density lipoprotein cholesterol levels and slightly higher FT3 and FT4 levels with no difference in TSH (Supplementary Table 1).

Prevalence of mild SCH and outcomes at the end of follow-up

At baseline, thyroid function tests were performed on 10,795 subjects among 11,000 participants, and 1,088 subjects had mild SCH (10.1%). After applying the exclusion criteria, 595 subjects were eligible, 90 subjects were excluded during the follow-up, finally 505 subjects were enrolled in our analysis (Fig. 1). Of 505 subjects with baseline mild SCH, 43.8% (n = 221) had persistent SCH, 49.7% (n = 251) had normalization of thyroid function, and 3.4% (n = 17) had OH at the end of follow-up. Other outcomes included 1 case of hyperthyroidism, 12 combinations of decreased FT4 levels and normal TSH levels and 3 combinations of increased FT4 levels and normal TSH levels. The rate of other outcomes revealed no difference between mild SCH cases and euthyroid controls (3.2% *vs*. 3.6%, p = 0.618), and the rate of progression to OH was significantly higher for cases with mild SCH than for euthyroid subjects (3.4% *vs*. 0.37%, p <0.001) (Data not shown).

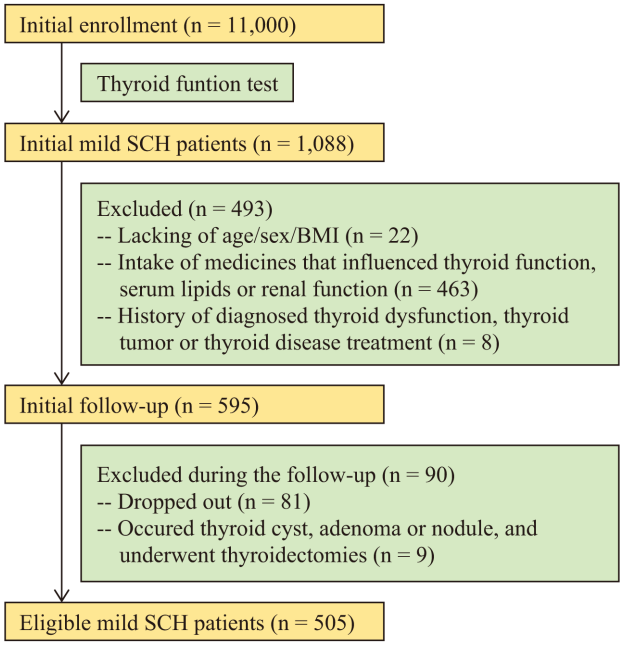

Fig. 1 Flowchart of enrollment process

Abbreviations: SCH, subclinical hypothyroidism; BMI, body mass index.

Baseline characteristics of the study population

Baseline characteristics of individuals with different thyroid function (euthyroid, SCH or OH) at the end of follow-up are presented in Table 1. Compared to patients who would revert to euthyroidism, patients with persistent SCH were significantly older and had higher TSH and lower FT3 and FT4 levels. Additionally, patients with OH had higher TSH than euthyroidism group and significantly lower baseline FT4 levels and a higher positive rate of TPOAb than the other two groups. The positive rate of TPOAb in 505 subjects accomplished the cohort was 19.8% (Supplementary Table 1). Besides, we inquired menstrual conditions in 294 subjects and found the proportion of menopausal subjects was similar among 3 groups ($p = 0.153$, data not shown).

Regression model

We performed logistic regression analysis to investigate factors associated with progression or resolution (Table 2 and Table 3). Age, sex, BMI, TC, GGT, CR, DM status, HT status, TSH and TPOAb status were substituted into equation simultaneous to find significant factors. Sex, BMI, GGT and the presence of diabetes or hypertension were not significantly related to the transition from mild SCH to euthyroidism or OH. Mild SCH patients with serum TC levels of 201.0-240.0 mg/dL or higher than 240.0 mg/dL had greater risks of developing OH than those with TC below 201.0 mg/dL (OR = 5.769 and 15.676, $p = 0.048$ and 0.006, respectively). Similarly, the incidence of developing OH was higher for those with positive TPOAb than patients with negative TPOAb (OR = 7.007, $p = 0.009$). Since limited cases in OH group and numerous variables might make our results suspect, we also performed a simplified regression model with 7 most interested variables and found similar results (Supplementary Table 2).

On the other hand, patients aged older than 60 years had a lower likelihood of reversion than those aged 50 years or younger (OR = 0.487, $p = 0.012$). Those with CR between 0.71-0.80 mg/dL or higher than 0.80 mg/dL

Table 1 Baseline characteristics of subjects with euthyroidism, SCH or OH at the end of follow-up

Characteristics	Euthyroidism (n=251)	SCH (n=221)	OH (n=17)	*p* value
Age, yr, mean (SD)	54.20 (7.92)	57.27 (8.74) [b]	55.94 (10.09)	**<0.001**
Female, n (%)	187 (74.5)	168 (76.0)	12 (70.6)	0.860
BMI, kg/m^2, mean (SD)	25.42 (3.62)	25.26 (4.21)	26.91 (4.40)	0.247
TC, mg/dL, mean (SD)	199.9 (42.9)	198.4 (39.1)	205.7 (40.2)	0.766
TG, mg/dL, median (IQR)	110.7 (90.3)	104.5 (77.1)	94.8 (47.8)	0.377
LDL-C, mg/dL, mean (SD)	117.2 (33.6)	116.2 (31.3)	117.2 (30.2)	0.946
HDL-C, mg/dL, mean (SD)	56.5 (13.5)	56.5 (13.9)	60.7 (15.9)	0.439
FPG, mg/dL, mean (SD)	112.8 (33.0)	114.4 (34.6)	128.1 (51.2)	0.206
HbA1c, %, mean (SD)	6.32 (1.39)	6.20 (1.12)	6.75 (1.86)	0.184
SBP, mm Hg [a], mean (SD)	139.55 (20.52)	140.95 (22.32)	138.94 (18.46)	0.754
DBP, mm Hg, mean (SD)	82.13 (11.79)	80.65 (12.07)	78.29 (12.88)	0.234
ALT, IU/L, mean (SD)	18.48 (10.93)	17.49 (9.07)	19.71 (7.52)	0.446
AST, IU/L, mean (SD)	22.20 (7.92)	21.86 (7.34)	24.24 (10.17)	0.464
GGT, IU/L, median (IQR)	19.00 (13.00)	18.00 (11.00)	18.00 (9.00)	0.704
Cr, mg/dL, mean (SD)	0.72 (0.13)	0.74 (0.13)	0.73 (0.08)	0.467
DM, n (%)	65 (25.9)	62 (28.1)	5 (29.4)	0.848
HT, n (%)	113 (45.0)	111 (50.2)	8 (47.1)	0.528
FT3, pg/mL, mean (SD)	3.19 (0.47)	3.05 (0.42) [b]	3.11 (0.32)	**0.004**
FT4, ng/dL, mean (SD)	1.24 (0.15)	1.20 (0.14) [b]	1.06 (0.10) [bc]	**<0.001**
TSH, mIU/L, median (IQR)	4.88 (1.07)	5.56 (1.81) [b]	5.46 (2.03) [b]	**<0.001**
TPOAb positive, n (%)	40 (15.9)	41 (18.6)	8 (47.1) [bc]	**0.006**

[a] Multiply 0.133 to convert values to kPa. [b] Differ from euthyroidism group and [c] differ from SCH group significantly in multiple comparisons. Abbreviations: SCH, subclinical hypothyroidism; OH, overt hypothyroidism; SD, standard deviation; IQR, interquartile range; BMI, body mass index; TC, total cholesterol; TG, total triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; DM, diabetes mellitus; HT, hypertension; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

were less likely to normalize their thyroid function than those with CR of 0.65 mg/dL or lower (OR = 0.529 and 0.350, $p = 0.031$ and 0.004, respectively). Additionally, patients with TSH of 7.00 mIU/L or higher also were less likely to revert to euthyroidism (OR = 0.192, $p < 0.001$). As we did not employ cut points with clinical significance for CR and GGT, we utilized these two parameters as continuous variables in each logistic analysis as well, and found similar results (Supplementary Table 3 and Supplementary Table 4).

Discussion

The present study, to the best of our knowledge, is the first prospective population-based cohort study of the natural history of mild SCH in the Chinese population. Our study revealed that nearly half of mild SCH subjects reverted to euthyroidism during a 3-year period. Furthermore, in addition to TSH and TPOAb status, lipid profiles and renal function may influence the course of the disease in some ways.

Table 2 Logistic regression analysis of basal characteristics related to progression of thyroid function

Variables	Progression to overt hypothyroidism			
	B	OR	95%CI	*p* value
Age, year				0.730
≤50	1			
51-60	-0.672	0.510	0.097-2.693	0.428
>60	-0.380	0.684	0.110-4.242	0.683
Sex				
Male	1			
Female	-1.910	0.148	0.016-1.371	0.093
BMI, kg/m^2				0.052
<24	1			
24.00-27.99	-1.066	0.344	0.047-2.500	0.292
≥28.00	1.249	3.486	0.582-20.877	0.171
TC, mg/dL				**0.020**
<201.0	1			
201.0-240.0	1.753	5.769	1.018-32.690	**0.048**
>240.0	2.752	15.676	2.227-110.367	**0.006**
GGT, IU/L				0.522
≤14.00	1			
14.01-18.00	-0.456	0.634	0.102-3.942	0.625
18.01-25.00	-1.108	0.330	0.042-2.618	0.294
>25.00	-1.777	0.169	0.014-2.040	0.162
CR, mg/dL				0.873
≤0.65	1			
0.66-0.71	0.292	1.339	0.239-7.489	0.739
0.72-0.80	0.258	0.773	0.105-5.700	0.800
>0.80	-0.501	0.606	0.056-6.505	0.679
DM				
No	1			
Yes	-0.720	0.487	0.086-2.759	0.416
HT				
No	1			
Yes	-1.185	0.306	0.071-1.308	0.110
TSH, mIU/L				
<7.00	1			
≥7.00	-0.078	0.925	0.198-4.315	0.921
TPOAb				
Negative	1			
Positive	1.947	7.007	1.620-30.297	**0.009**

All variables were substituted into regression model simultaneously to scan predictors. Abbreviations: OR, odds rate; CI, confidence interval; BMI, body mass index; TC, total cholesterol; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; DM, diabetes mellitus; HT, hypertension; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

Table 3 Logistic regression analysis of basal characteristics related to normalization of thyroid function

Variables	Normalization of thyroid function			
	B	OR	95%CI	*p* value
Age, year				**0.028**
≤50	1			
51-60	-0.168	0.845	0.517-1.383	0.504
>60	-0.719	0.487	0.279-0.851	**0.012**
Sex				
Male	1			
Female	-0.457	0.633	0.347-1.157	0.137
BMI, kg/m^2				0.733
<24	1			
24.00-27.99	0.150	1.162	0.728-1.854	0.530
≥28.00	-0.034	0.967	0.544-1.720	0.909
TC, mg/dL				0.501
<201.0	1			
201.0-240.0	-0.037	0.964	0.598-1.553	0.880
>240.0	0.329	1.389	0.751-2.570	0.295
GGT, IU/L				0.529
≤14.00	1			
14.01-18.00	0.267	1.306	0.736-2.317	0.361
18.01-27.00	0.396	1.486	0.827-2.669	0.185
>27.00	0.443	1.558	0.794-3.059	0.198
CR, mg/dL				**0.035**
≤0.65	1			
0.66-0.70	-0.469	0.626	0.353-1.109	0.109
0.71-0.80	-0.637	0.529	0.297-0.943	**0.031**
>0.80	-1.049	0.350	0.170-0.722	**0.004**
DM				
No	1			
Yes	-0.034	0.966	0.605-1.543	0.886
HT				
No	1			
Yes	-0.139	0.870	0.572-1.325	0.517
TSH, mIU/L				
<7.00	1			
≥7.00	-1.648	0.192	0.098-0.378	**<0.001**
TPOAb				
Negative	1			
Positive	0.031	1.032	0.603-1.764	0.909

All variables were substituted into regression model simultaneously to scan predictors. Abbreviations: OR, odds rate; CI, confidence interval; BMI, body mass index; TC, total cholesterol; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; DM, diabetes mellitus; HT, hypertension; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

A total of 505 mild SCH patients completed the present study, more than several previous studies on this topic [6-11]. Compared with other studies, we included individuals aged 40-years or older instead of only elderly people in both sexes [6-11]. Furthermore, the population prevalence of mild SCH is 10.1%, within the range of 4% - 20% described by previous articles [4]. This suggests that our study, at least to some extent, represented the natural history of mild SCH in the middle-aged and elderly Chinese population.

In our 3-year cohort study, we found that subjects with SCH had significantly higher risks of progression to OH compared to euthyroid subjects. An investigation conducted in Japan [8] drew a similar conclusion, but risks were higher either in SCH subjects (7.0%) or euthyroid subjects (1.6%) than in our study. The difference might partly result from the higher baseline TSH levels in their study population, as they enrolled some severe SCH patients. The difference might also result from the generally higher iodine intake in Japan. Huber *et al*. [11] revealed a high degree of progression (28%) in a study of 82 women. Their study included patients who received thyroid disease treatment such as thyroidectomy and radioiodine therapy, and the TPOAb positive rate was 51%. Both these factors could influence outcomes to some extent. Previous studies have documented well that the risk of progression of thyroid function was positively correlated with baseline TSH concentrations as well as TPOAb status [6-8, 10, 11, 20]. But for mild SCH population only, Rosário *et al*. found that only initial TSH was a predictor of the need for L-T4 therapy [6, 7]. When it comes to our study, the presence of baseline TPOAb still had a significant positive association with progression of thyroid function in mild SCH, whereas baseline TSH did not. As the patients treated with L-T4 included not only OH patients, but also severe SCH patients in Rosário's study, the inconsistent conclusion might result from the different prognostic value of TSH in OH and severe SCH. Also, their study reported a population initial TSH value at 7.4 mIU/L, which means the patients we enrolled were relatively milder compared with previous studies focused on this topic [7]. Furthermore, the number of subjects who progressed was relatively small in our analysis. For these reasons, TSH levels did not reveal a relationship with progression to OH in our analysis. For all this, our results implied that TSH, compared to TPOAb, might not play a very important role in the natural history of our uncomplicated mild SCH population.

A novel finding of our study was that patients with higher serum TC had significantly higher risk of progression to OH. It has been clarified by previous studies that thyroid hormones regulate lipid metabolism, including TC. Furthermore, lipid disorders including elevated cholesterol were observed even in SCH patients [21]. Previous studies in our laboratory suggested that the independent effect of TSH on hepatic cholesterol metabolism might be the mechanism involved [22-24]. On the other hand, lipid profile can somehow influence the prevalence of SCH. Bindels *et al*. [25] reported a dramatic increase of SCH prevalence from 4.0% in middle-aged women with total plasma cholesterol <193.3 mg/dL to 10.3% in those with TC >309.4 mg/dL. They also observed a trend of increased OH prevalence as TC rose in both sexes. In regression analysis of TC, we used 201.0 mg/dL and 240.0 mg/dL as cut-off points that were also used to evaluate cardiovascular risk [26]. Our study demonstrated that mild SCH subjects with TC between 201.0-240.0 mg/dL had 5 times greater risk of developing OH compared with patients with TC <201.0 mg/dL. And the risk was 15 times greater in patients with TC >240.0 mg/dL. These results suggested the possibility that TC might be a promising predictor and the control of TC levels may benefit mild SCH patients in a very efficient way. As we known, estrogen can regulate lipids metabolism. However, the similar proportion of menopausal subjects among 3 groups indicated that estrogen levels might not be involved in the underlying mechanism. Zhao *et al*. found that thyroid might be a target organ for the deposition of triglyceride, which evoked the hypothesis that thyroid was the target organ of cholesterol as well [15]. Previous studies found that cholesterol inhibited the activity of extracellular signal-regulated kinase (ERK) [27], and ERK signaling pathway can up regulate the phosphorylation of thyroid transcription factor 1 (TTF-1) [28], which is a thyroid specific transcription factor and essential for the expression of thyroid hormone synthesis-related molecules thyroglobulin (TG), thyroid peroxidase (TPO) and sodium/iodide symporter (NIS) [29]. Besides, previous studies demonstrated that cholesterol was a major component of endoplasmic reticulum (ER), and excess cholesterol could induce ER stress [30, 31], which was associated with thyroid dysfunction [32]. Thus, the abnormal synthesis of thyroid hormones caused by the above mechanism contributed to the hypothyroidism progression. As this is a clinical study, it is difficult for us to find the

real cause of hypothyroidism progression, which is a limitation of our study. We necessitate further research to verify our findings and hypothesis

Nearly half of our individuals with mild SCH (49.7%) experienced a normalization of thyroid function during a 3-year period in our study. The rate of spontaneous normalization in this study was similar to that reported in Japan (53.5% over 4.2 years), but was higher than rates reported in the USA (35% over 2 years) [6], Brazil (22.8% over 5 years) [7] and Spain (37.4% over 0.5-6 years) [10]. Huber *et al*. [11] even reported a 4% rate of regression in a population that included patients with diagnosed thyroid diseases. Although these results are less comparable because of different characteristics of participants in the different studies, we found a regression rate higher than most previous studies in our study, which was the only one to enroll only patients with mild SCH from both sexes. Based on previous studies and expert reviews, we used 7.0 mIU/L of TSH as cut-off points [1, 13] and found that higher TSH concentrations significantly impaired the likelihood of TSH normalization in our logistic regression analysis, similar to previous studies [6, 8, 10]. Accordingly, the generally lower TSH levels among our cases compared with previous studies may be one of the reasons that TSH normalization occurred in a high proportion of our patients. We also found results consistent with Somwaru *et al*. [6] and Rosário *et al*. [7] in that spontaneous normalization status was similar among patients with positive TPOAb and those without. The difference is that we found that age also had negative correlation with TSH reversion to normal in our analysis. As the age range in our study was between 40 and 77-years, the wider range and younger age compared with previous studies also contributed to the different results and the higher regression rate in the present study [6, 10].

The relationship between thyroid function and renal function has frequently been discussed in previous articles. Asvold *et al*. [33] found that low thyroid function, characterized by high TSH levels even within the normal range, were associated with reduced renal function expressed as CR level or estimated glomerular filtration rate (GFR). On the other hand, patients with CKD had an increased prevalence of hypothyroidism and SCH [34]. Furthermore, we stratified patients based on the quartiles of CR level and analyzed the association between serum CR and the natural history of mild SCH in a longitudinal study. The results revealed that patients with higher baseline CR concentrations within the normal reference range were dramatically less likely to revert to euthyroidism. The results were similar when we utilizing CR as continuous variable. However, CR levels were not associated with progression to OH in the present study. This may be because the slightly increased CR within the normal range did not affect thyroid function as much as CKD did. Our results suggested that renal function expressed as serum CR concentration somehow correlated with the outcome of mild SCH. One plausible mechanism is that patients with impaired renal function (characterized by higher CR) excreted less iodine into urine and then made the patients more susceptible to excess iodide, which may influence thyroid function, although it should be verified in mild SCH patients [1, 35].

It was reported that TSH levels increased with age and indicated an overestimation of SCH prevalence in older people [36]. A recent study provided a method to determine a population-specific TSH reference range [37]. However, we had no access to a Chinese-specific range at present. Furthermore, to our knowledge, there is no previous population-based study on this topic using an age-specific reference range, as this might influence the risks of various outcomes [8]. Therefore, we employed a common TSH reference range of 0.27-4.2 mIU/L.

Our study has several limitations. First, we estimated thyroid function based on a single thyroid function test at each visit. Although Andersen *et al*. [38] monitored thyroid function of 16 men monthly and found the individual variations were narrow, it is possible that some of our participants have been misclassified, and our results have gotten potential bias. Besides, as we discussed earlier, one of the problems with this analysis is the relatively small number of patients that progressed to OH (n=17), which meant that our result was not such robust. Although our simplified regression model found similar results, which suggested that our findings at least might provide implications about the effect of total cholesterol on hypothyroidism progression, we need a larger study to verify our results. Additionally, although we communicated with participants by telephone every 2 months and conducted physical examinations convenient for participants, 90 mild SCH patients failed to complete the cohort. However, we found that baseline characteristics were similar between subjects who accomplished the cohort and these 90 subjects, which meant that our results

were representative to some extent. Finally, we did not perform ultrasound tests, which had been reported to have a considerable predictive value in outcomes of SCH, because of the lack of ultrasound scanners and experienced doctors [9].

In conclusion, our present study demonstrated that nearly half of mild SCH patients revert to euthyroidism. In mild SCH patients, TPOAb and TC seem to be more important predictors of progression to OH than initial TSH, whereas high baseline TSH or CR were negative correlated with reversion to euthyroidism. The prognostic value of TC and CR in mild SCH should be considered. Patients with high TC or CR might need more frequent monitoring of thyroid function. Future studies should exam the possible benefits of L-thyroxine treatment in patients with high TC or CR levels.

Acknowledgements

We are grateful to all participants and grassroots doctors for cooperation. This research was supported by grants from the National Natural Science Foundation (81430020, 81230018 and 81270869), funds from department of Science & Technology of Shandong (2016GSF201013, 2016GSF201015), and Research Special Funds for Public Welfare Projects (201402005 and 201502007).

Disclosure

None of the authors has any potential conflict of interest associated with this research.

Supplementary Table 1 Baseline characteristics between subjects who accomplished the cohort and those did not

Characteristics	Accomplished (n=505)	Un-accomplished (n=90)	*p* value
Age, y, mean (SD)	55.52 (8.51)	56.26 (9.46)	0.458
Female, n (%)	382 (75.6)	63 (70.0)	0.256
BMI, kg/m^2, mean (SD)	25.42 (3.94)	25.05 (5.18)	0.434
TC, mg/dL, mean (SD)	199.5 (41.0)	191.0 (41.8)	0.064
TG, mg/dL, median (IQR)	107.2 (77.1)	106.3 (82.4)	0.890
LDL-C, mg/dL, mean (SD)	116.8 (32.5)	114.8 (33.6)	0.614
HDL-C, mg/dL, mean (SD)	56.5 (13.5)	49.5 (12.0)	**<0.001**
FPG, mg/dL, mean (SD)	114.2 (36.2)	115.0 (35.1)	0.871
HbA1c, %, mean (SD)	6.28 (1.28)	6.34 (1.26)	0.701
SBP, mm Hg [a], mean (SD)	140.04 (21.15)	141.25 (23.35)	0.625
DBP, mm Hg, mean (SD)	81.33 (11.88)	82.51 (11.75)	0.388
ALT, IU/L, mean (SD)	18.03 (9.92)	17.43 (10.98)	0.608
AST, IU/L, mean (SD)	22.19 (7.72)	22.39 (9.85)	0.855
GGT, IU/L, median (IQR)	18.00 (12.00)	18.00 (18.50)	0.402
Cr, mg/dL, mean (SD)	0.73 (0.13)	0.75 (0.15)	0.142
DM, n (%)	134 (26.5)	29 (32.2)	0.265
HT, n (%)	239 (47.3)	46 (51.1)	0.508
FT3, pg/mL, mean (SD)	3.12 (0.45)	3.02 (0.48)	**0.039**
FT4, ng/dL, mean (SD)	1.21 (0.15)	1.18 (0.14)	**0.037**
TSH, mIU/L, median (IQR)	5.17 (1.61)	5.24 (1.83)	0.586
TPOAb positive, n (%)	94 (19.8)	17 (18.9)	0.837

[a] Multiply 0.133 to convert values to kPa. Abbreviations: SD, standard deviation; IQR, interquartile range; BMI, body mass index; WC, waist; TC, total cholesterol; TG, total triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; DM, diabetes mellitus; HT, hypertension; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

Supplementary Table 2 Simplified logistic regression analysis of basal characteristics related to progression of thyroid function

Variables	Progression to overt hypothyroidism			
	B	OR	95%CI	*p* value
Age, year				0.620
≤50	1			
51-60	-0.699	0.497	0.106-2.335	0.376
>60	-0.672	0.511	0.106-2.463	0.403
Sex				
Male	1			
Female	-1.858	0.156	0.024-1.026	0.053
TC, mg/dL				**0.047**
<201.0	1			
201.0-240.0	1.417	4.123	0.873-19.473	0.074
>240.0	2.161	8.682	1.522-49.518	**0.015**
GGT, IU/L				0.687
≤14.00	1			
14.01-18.00	-0.079	0.924	0.192-4.435	0.921
18.01-25.00	-0.273	0.761	0.144-4.031	0.748
>25.00	-1.245	0.288	0.034-2.410	0.251
CR, mg/dL				0.595
≤0.65	1			
0.66-0.71	0.752	2.121	0.376-11.965	0.394
0.72-0.80	0.017	1.018	0.157-6.588	0.985
>0.80	-0.392	0.676	0.080-65.714	0.719
TSH, mIU/L				
<7.00	1			
≥7.00	-0.359	0.698	0.154-3.160	0.641
TPOAb				
Negative	1			
Positive	2.069	7.915	2.008-31.197	**0.003**

All variables were substituted into regression model simultaneously to scan predictors. Abbreviations: OR, odds rate; CI, confidence interval; TC, total cholesterol; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

Supplementary Table 3 Logistic regression analysis of basal characteristics related to progression of thyroid function utilizing continuous GGT and CR

Variables	Progression to overt hypothyroidism			
	B	OR	95%CI	*p* value
Age, year				0.718
≤50	1			
51-60	-0.681	0.506	0.097-2.630	0.418
>60	-0.482	0.618	0.108-3.517	0.587
Sex				
Male	1			
Female	-1.841	0.159	0.018-1.368	0.094
BMI, kg/m^2				0.054
<24	1			
24.00-27.99	-1.146	0.318	0.048-2.093	0.233
≥28.00	1.025	2.788	0.596-13.032	0.193
TC, mg/dL				**0.031**
<201.0	1			
201.0-240.0	1.488	4.428	0.890-22.032	0.069
>240.0	2.540	12.677	1.871-85.883	**0.009**
GGT, IU/L	-0.036	0.964	0.895-1.040	0.347
CR, mg/dL	-0.032	0.969	0.896-1.048	0.425
DM				
No	1			
Yes	-0.585	0.557	0.107-2.908	0.488
HT				
No	1			
Yes	-1.190	0.304	0.077-1.200	0.089
TSH, mIU/L				
<7.00	1			
≥7.00	-0.120	0.887	0.197-3.984	0.875
TPOAb				
Negative	1			
Positive	1.848	6.350	1.565-25.764	**0.010**

All variables were substituted into regression model simultaneously to scan predictors. Abbreviations: OR, odds rate; CI, confidence interval; BMI, body mass index; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; DM, diabetes mellitus; HT, hypertension; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

Supplementary Table 4 Logistic regression analysis of basal characteristics related to normalization of thyroid function utilizing continuous GGT and CR

Variables	Normalization of thyroid function			
	B	OR	95%CI	*p* value
Age, year				**0.020**
≤50	1			
51-60	-0.145	0.865	0.532-1.407	0.558
>60	-0.733	0.481	0.277-0.835	**0.009**
Sex				
Male	1			
Female	-0.444	0.642	0.363-1.133	0.126
BMI, kg/m^2				0.776
<24	1			
24.00-27.99	0.159	1.172	0.741-1.854	0.497
≥28.00	0.037	1.037	0.597-1.802	0.896
TC, mg/dL				0.480
<201.0	1			
201.0-240.0	0.035	1.036	0.656-1.636	0.880
>240.0	0.365	1.441	0.790-2.628	0.234
GGT, IU/L	0.003	1.003	0.993-1.014	0.531
CR, mg/dL	-0.023	0.978	0.956-0.999	**0.043**
DM				
No	1			
Yes	-0.012	0.988	0.623-1.569	0.961
HT				
No	1			
Yes	-0.120	0.887	0.571-1.344	0.571
TSH, mIU/L				
<7.00	1			
≥7.00	-1.606	0.201	0.103-0.390	**<0.001**
TPOAb				
Negative	1			
Positive	0.044	1.045	0.616-1.775	0.869

All variables were substituted into regression model simultaneously to scan predictors. Abbreviations: OR, odds rate; CI, confidence interval; BMI, body mass index; GGT, gamma-glutamyl transpeptidase; Cr, creatinine; DM, diabetes mellitus; HT, hypertension; TSH, thyrotropin; TPOAb, thyroid peroxidase antibody.

References

1. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, *et al*. (2004) Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA* 291: 228-238.
2. Pearce SH, Brabant G, Duntas LH, Monzani F, Peeters RP, *et al*. (2013) 2013 ETA Guideline: Management of Subclinical Hypothyroidism. *Eur Thyroid J* 2: 215-228.
3. Biondi B (2012) Natural history, diagnosis and management of subclinical thyroid dysfunction. *Best Pract Res Clin Endocrinol Metab* 26: 431-446.
4. Cooper DS, Biondi B (2012) Subclinical thyroid disease. *Lancet* 379: 1142-1154.
5. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC (2000) The Colorado thyroid disease prevalence study. *Arch Intern Med* 160: 526-534.
6. Somwaru LL, Rariy CM, Arnold AM, Cappola AR

(2012) The natural history of subclinical hypothyroidism in the elderly: the cardiovascular health study. *J Clin Endocrinol Metab* 97: 1962-1969.
7. Rosario PW, Carvalho M, Calsolari MR (2016) Natural history of subclinical hypothyroidism with TSH ≤10 mIU/l: a prospective study. *Clin Endocrinol (Oxf)* 84: 878-881.
8. Imaizumi M, Sera N, Ueki I, Horie I, Ando T, *et al*. (2011) Risk for progression to overt hypothyroidism in an elderly Japanese population with subclinical hypothyroidism. *Thyroid* 21: 1177-1182.
9. Rosario PW, Bessa B, Valadao MM, Purisch S (2009) Natural history of mild subclinical hypothyroidism: prognostic value of ultrasound. *Thyroid* 19: 9-12.
10. Díez JJ, Iglesias P (2004) Spontaneous Subclinical Hypothyroidism in Patients Older than 55 Years: An Analysis of Natural Course and Risk Factors for the Development of Overt Thyroid Failure. *J Clin Endocrinol Metab* 89: 4890-4897.
11. Huber G, Staub JJ, Meier C, Mitrache C, Guglielmetti M, *et al*. (2002) Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. *J Clin Endocrinol Metab* 87: 3221-3226.
12. Parle JV, Franklyn JA, Cross KW, Jones SC, Sheppard MC (1991) Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. *Clin Endocrinol (Oxf)* 34: 77-83.
13. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, *et al*. (2010) Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *JAMA* 304: 1365-1374.
14. Ning G (2012) Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) study. *J Diabetes* 4: 172-173.
15. Zhao M, Tang X, Yang T, Zhang B, Guan Q, *et al*. (2015) Lipotoxicity, a potential risk factor for the increasing prevalence of subclinical hypothyroidism? *J Clin Endocrinol Metab* 100: 1887-1894.
16. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, *et al*. (2000) Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 23: 295-301.
17. Xu Y, Wang L, He J, Bi Y, Li M, *et al*. (2013) Prevalence and Control of Diabetes in Chinese Adults. *JAMA* 310: 948-959.
18. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, *et al*. (2014) 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA* 311: 507-520.
19. Goodman NF, Cobin RH, Ginzburg SB, Katz IA, Woode DE (2011) American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the diagnosis and treatment of menopause. *Endocr Pract* 17: 949-954.
20. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, *et al*. (1995) The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin Endocrinol (Oxf)* 43: 55-68.
21. Duntas LH, Brenta G (2012) The effect of thyroid disorders on lipid levels and metabolism. *Med Clin North Am* 96: 269-281.
22. Tian L, Song Y, Xing M, Zhang W, Ning G, *et al*. (2010) A novel role for thyroid-stimulating hormone: up-regulation of hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase expression through the cyclic adenosine monophosphate/protein kinase A/cyclic adenosine monophosphate-responsive element binding protein pathway. *Hepatology* 52: 1401-1409.
23. Wang F, Tan Y, Wang C, Zhang X, Zhao Y, *et al*. (2012) Thyroid-Stimulating Hormone Levels within the Reference Range Are Associated with Serum Lipid Profiles Independent of Thyroid Hormones. *J Clin Endocrinol Metab* 97: 2724-2731.
24. Xu C, Yang X, Liu W, Yuan H, Yu C, *et al*. (2012) Thyroid stimulating hormone, independent of thyroid hormone, can elevate the serum total cholesterol level in patients with coronary heart disease: a cross-sectional design. *Nutr Metab (Lond)* 9: 44.
25. Bindels AJ, Westendorp RG, Frolich M, Seidell JC, Blokstra A, *et al*. (1999) The prevalence of subclinical hypothyroidism at different total plasma cholesterol levels in middle aged men and women: a need for case-finding? *Clin Endocrinol (Oxf)* 50: 217-220.
26. D'Agostino RS, Vasan RS, Pencina MJ, Wolf PA, Cobain M, *et al*. (2008) General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 117: 743-753.
27. Wang PY, Weng J, Anderson RG (2005) OSBP is a cholesterol-regulated scaffolding protein in control of ERK 1/2 activation. *Science* 307: 1472-1476.
28. Missero C, Pirro MT, Di Lauro R (2000) Multiple Ras Downstream Pathways Mediate Functional Repression of the Homeobox Gene Product TTF-1. *Mol Cell Biol* 20: 2783-2793.
29. Ke CC, Hsieh YJ, Hwu L, Wang FH, Chen FD, *et al*. (2011) Evaluation of lentiviral-mediated expression of sodium iodide symporter in anaplastic thyroid cancer and the efficacy of in vivo imaging and therapy. *J Oncol* 2011: 178967.
30. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, *et al*. (2003) The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol* 5: 781-792.
31. Li Q, Liu Z, Guo J, Chen J, Yang P, *et al*. (2009) Cholesterol overloading leads to hepatic L02 cell damage through activation of the unfolded protein response. *Int J Mol Med* 24: 459-464.

32. Park S, You KH, Shong M, Goo TW, Yun EY, *et al*. (2005) Overexpression of ERp29 in the thyrocytes of FRTL-5 cells. *Mol Biol Rep* 32: 7-13.
33. Asvold BO, Bjoro T, Vatten LJ (2010) Association of thyroid function with estimated glomerular filtration rate in a population-based study: the HUNT study. *Eur J Endocrinol* 164: 101-105.
34. Lo JC, Chertow GM, Go AS, Hsu CY (2005) Increased prevalence of subclinical and clinical hypothyroidism in persons with chronic kidney disease. *Kidney Int* 67: 1047-1052.
35. Sato K, Okamura K, Yoshinari M, Kuroda T, Ikenoue H, *et al*. (1992) Reversible primary hypothyroidism and elevated serum iodine level in patients with renal dysfunction. *Acta Endocrinol (Copenh)* 126: 253-259.
36. Surks MI, Hollowell JG (2007) Age-Specific Distribution of Serum Thyrotropin and Antithyroid Antibodies in the U.S. Population: Implications for the Prevalence of Subclinical Hypothyroidism. *J Clin Endocrinol Metab* 92: 4575-4582.
37. Boucai L, Hollowell JG, Surks MI (2011) An Approach for Development of Age-, Gender-, and Ethnicity-Specific Thyrotropin Reference Limits. *Thyroid* 21: 5-11.
38. Andersen S, Pedersen KM, Bruun NH, Laurberg P (2002) Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab* 87: 1068-1072.