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Moderating Role of TSHR and PTPN22 Gene Polymorphisms in Effects of Excessive Fluoride on Thyroid: a School-Based Cross-Sectional Study

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Abstract

We aimed to investigate the relationship between the effects excessive of fluoride on thyroid health in children and the moderating role of thyroid stimulating hormone receptor (TSHR) or protein tyrosine phosphatase nonreceptor-22 (PTPN22) gene polymorphisms. Four hundred thirteen children (141 with dental fluorosis and 198 boys) were enrolled from both historical endemic and non-endemic areas of fluorosis in Tianjin, China. The fluoride exposure levels, thyroid health indicators, and TSHR (rs2268458) and PTPN22 (rs3765598) polymorphisms were examined. Multiple logistic models were applied to evaluate the relationship between dental fluorosis and thyroid abnormalities. Children over 9 year old with dental fluorosis have lower FT_4 and TGAb levels and thyroid volume and higher TPOAb levels (all $P < 0.05$). In overall participants, children with dental fluorosis were more likely to have thyroid antibody single positive issues (adjusted $P = 0.039$) and less likely to have a goiter according to age or body surface area (age or BSA) (adjusted $P = 0.003$); In the TSHR (rs2268458) SNP = CC/CT or PTPN22 (rs3765598) SNP = CC subgroup, dental fluorosis may cause thyroid antibody single positive (adjusted $P = 0.036$; adjusted $P = 0.002$); in the TSHR (rs2268458) SNP = TT or PTPN22 (rs3765598) SNP = CC subgroup, dental fluorosis may protect children from goiter (age or BSA) (adjusted $P = 0.018$; adjusted $P = 0.013$). Excessive fluoride may induce thyroid antibody single positive and reduce goiter in children. Heterogeneity exists in the relationship between excessive fluoride and thyroid antibody single positive or goiter issues across children carrying different TSHR (rs2268458) or PTPN22 (rs3765598) genotypes.

Keywords Dental fluorosis · Thyroid · TSHR · PTPN22 · Polymorphisms

Introduction

Thyroid diseases can markedly affect health, particularly hyperthyroidism and hypothyroidism, which may lead to devastating related outcomes in all human bodies [1]. For the past two decades, the thyroid disease spectrum has changed dramatically. For example, in mainland China, hyperthyroidism and goiter have decreased significantly, but subclinical hypothyroidism and thyroid nodules have increased [2]. The risk factors for thyroid disease have also been investigated. Iodine deficiency, iodine excess, female sex, and alcohol consumption are risk factors for hyperthyroidism [1]. Iodine excess is a risk factor for subclinical hypothyroidism [2]. Older age, female sex, and high education level may be risk factors for thyroid nodules [3]. Interestingly, few studies have explored the relationship between excessive fluoride and thyroid disease.

Fluorine is an essential trace element for the human body, and is widely distributed in the environment. The intake of fluorine in the human body occurs mainly through drinking groundwater with excessive fluoride [4, 5]. Fluorosis easily occurs when the fluoride content in water is higher than 1.0 mg/L [6]. Presently, many animal experiments have found that excessive fluoride can affect the function and structure of the thyroid [7–9]. However, epidemiological investigations on the effect of excessive fluoride on thyroid function and

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structure have reported inconsistent results [10, 11]. Considering that approximately 200 million people in the worldwide are exposed to excessive fluoride [5], it is of public health significance to study the effects of excessive fluoride on thyroid health.

Disease is often caused by a combination of environmental factors and genetic susceptibility. Whether gene polymorphisms of key factors of thyroid function play a regulatory role in the relationship between excessive fluoride and thyroid diseases is also our concern. Thyroid stimulating hormone receptor (TSHR) is the key factor maintaining normal thyroid function. Studies have confirmed that TSHR gene mutation is related to low serum TSH levels [12] and toxic thyroid adenomas [13], and the TSHR (rs2268458) polymorphism is associated with autoimmune thyroid disease (AITD) in the UK population [14]. Additionally, the protein tyrosine phosphatase nonreceptor-22 (PTPN22) gene is gene most closely related to AITD, and polymorphisms of different PTPN22 gene loci have different effects on thyroid diseases [15–17]. However, the relationship between the PTPN22 (rs3765598) polymorphism and thyroid diseases has not been reported. Therefore, we chose TSHR (rs2268458) and PTPN22 (rs3765598) to explore their direct effects on the thyroid and their moderating role in the effects of excessive fluoride on thyroid.

In the present study, we selected children from water-borne fluoride excess areas and normal areas in Tianjin, China, to detect the internal exposure index of fluoride (dental fluorosis), thyroid function and structure, the single nucleotide polymorphisms (SNPs) of TSHR (rs2268458) and PTPN22 (rs3765598) to explore the effects of fluoride excess on thyroid health, and the moderating effects of these two gene polymorphisms in this process.

Materials and Methods

Study Population and Sampling

Chinese national drinking water standard (GB 5749-2006) stipulates that the concentration of fluoride in small centralized or decentralized water supply should not exceed 1.2 mg/L, so the area with fluoride concentration of > 1.2 mg/L is considered to be an excessive fluoride area. In our study, from 2018 to 2019, according to the water fluoride concentration data provided by Tianjin Centers for Disease Control and Prevention, all three schools in Dazhangzhuang town of Beichen District (including excessive fluoride and normal areas, mainly excessive fluoride area, with water fluoride concentration of 0.41–3.81 mg/L) and one central primary school in Xinkou town of Xiqing District (normal area, with a water fluoride concentration is 0.24–0.26 mg/L) were chosen. According to the data of children's urinary iodine published annually by the Tianjin Health Commission, Beichen and Xiqing are not iodine malnutrition areas. We then selected the corresponding grades using the cluster sampling method according to the preset age (7–12 years old), and randomly selected classes in each grade. With the consent of the guardians, 438 children were included in the study. After excluding children with incomplete information and noncompliant biomaterials, 413 children were analyzed and the selection process is shown in Fig. 1.

Anthropometry and Dental Fluorosis Detection

The height and weight of the children were examined using standard methods accurate to 1 cm and 0.1 kg respectively. The body mass index (BMI) = Weight (kg)/(height (cm)/100)^2, and the body surface area (BSA) = Weight (kg)$^{0.425}$*height (cm)$^{0.725}$*71.84*10^{-4}.

Dental fluorosis in children was confirmed by professional stomatologists according to the Chinese standard "diagnosis of dental fluorosis" (WS/T 208-2011), which was revised based on Dean's method. The standard divides dental fluorosis into normal, suspicious, extremely light, mild, moderate, and severe. To eliminate the subjective influence on the judgment of dental fluorosis, the normal and suspicious group were regarded as the "normal" group, and the remaining groups were regarded as the "dental fluorosis" group.

Measurement of Urinary Fluoride and Urinary Iodine Levels

Random urine was collected from all the subjects to detect urinary fluoride and urinary iodine. The urinary fluoride concentration was determined using fluoride ion-selective electrode method (Chinese standard WS/T 89-2015). The urinary iodine concentration was determined by inductively coupled plasma mass spectrometry method (WS/T 107.2-2006). Urinary iodine and fluoride detection was performed by KingMed Diagnostics (Guangzhou, Guangdong, China), one of the largest medical testing companies in China.

Measurement of Thyroid Function and Diagnosis of Thyroid Diseases

Three milliliters of non-anticoagulant venous blood was collected. The levels of free triiodothyronine (FT_3), free thyroxine (FT_4), thyroid stimulating hormone (TSH), thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TGAb) in serum were determined by electrochemiluminescence, which was also performed by KingMed Diagnostics (Guangzhou, Guangdong, China). The detection instruments and kits were purchased from Roche R&D Center (Shanghai China).

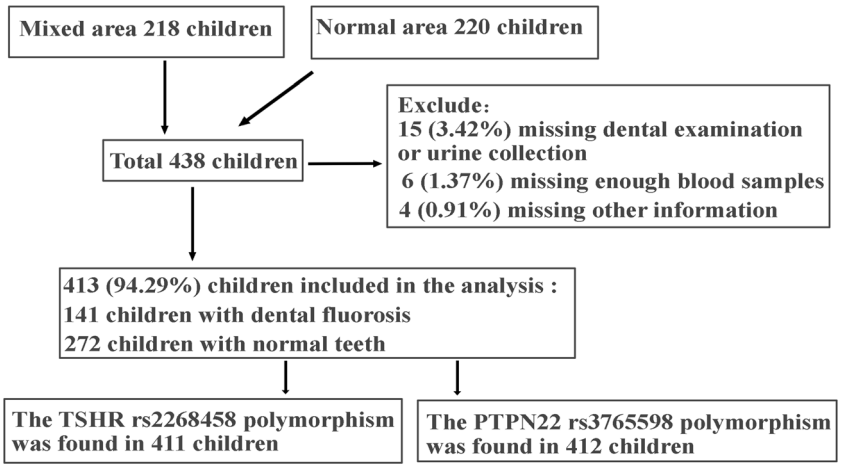

Fig. 1 Flowchart of the study

According to the manufacturer's instructions of the test kit, the reference ranges of the biochemical indexes of thyroid function were as follows: FT_3 5.42–7.93 pmol/L; FT_4 12.9–19.7 pmol/L; TSH 1.13–5.34 mIU/L; TGAb < 37 IU/mL; TPOAb < 18 IU/mL. The diagnosis of thyroid diseases was based on the study of Zhai et al. [18] as follows: overt hyperthyroidism (Ohyper): TSH < 1.13 mIU/L, FT_4 > 19.7 pmol/L, and/or FT_3 > 7.93 pmol/L; subclinical hyperthyroidism (Shyper): TSH < 1.13 mIU/L, normal FT_3 and FT_4; overt hypothyroidism (Ohypo): TSH > 5.34 mIU/L and FT_4 < 12.9 pmol/L; subclinical hypothyroidism (Shypo): TSH > 5.34 mIU/L and normal FT_4. Thyroid antibody positive: TGAb ≥ 37.0 IU/mL and/or TPOAb ≥ 18.0 IU/mL; double positive: TPOAb(+) and TGAb(+); single positive: TPOAb(+) or TgAb(+).

Thyroid Nodules and Volume Examination

Thyroid ultrasound examination was performed to detect thyroid nodules and volume by a professional B-ultrasound doctor using a B-type ultrasound diagnostic apparatus with a probe frequency of 7.5 MHZ. The child was lying on a chair with the head tilted back. The transverse section measured W; the longitudinal section measured L and D; and Tvol = $0.479*(W_{left}*L_{left}*D_{left+}\ W_{right}*L_{right}*D_{right})$. According to the thyroid volume of different ages or BSA, the goiter was evaluated. The age-related standard adopted the Chinese "Diagnostic Standards for Endemic Goiter" (WS 276-2007): the upper limits of the thyroid volume of normal children aged 7–12 years were 4.0, 4.5, 5.0, 6.0, 7.0, and 8.0 mL. BSA-related standards were recommended by WHO et al. [19] as follows: when the BSA range was 0.7–1.6, the upper limits of thyroid volume for boys were 2.62, 2.95, 3.32, 3.73, 4.20, 4.73, 5.32, 5.98, 6.73, and 7.57 mL; for girls were 2.56, 2.91, 3.32, 3.79, 4.32, 4.92, 5.61, 6.40, 7.29, and 8.32 mL. In the present study, an age-related goiter was named goiter (age); the BSA-related goiter was named goiter (BSA); the goiter that met both age- and BSA-related criteria was goiter (age and BSA); the goiter that met one of the criteria was goiter (age or BSA).

Detection of Genetic Polymorphisms

We collected 1 mL of venous anticoagulant blood, and used a blood DNA extraction kit (Tiangen Biotech (Beijing) Co., LTD) to extract DNA. Next, we used the Sequenom MassArray method to detect the SNPs of TSHR (rs2268458) and PTPN22 (rs3765598). This method mainly included the following steps: primer design, primer synthesis, DNA sample quality inspection, PCR amplification after primer dilution, SAP (shrimp alkaline phosphatase) reaction, single base extension termination reaction, mass spectrometry detection after resin purification, and data collection. The test was performed by Beijing Sequs Biotech Co. Ltd (Beijing, China). The primers used for TSHR (rs2268458) were as follows: 2nd-PCRP, ACG TTG GAT GAG CAC AAA GAG ACA CAC TGG; 1ST-PCRP, ACG TTG GAT GTC AAT GTG CGG GAG TTC TTC. The primers for PTPN22 (rs3765598) were as follows: 2nd-PCRP, ACG TTG GAT GGT AAA ATA AGG AAT GAA AGG; 1ST-PCRP, ACG TTG GAT GTG CTC TAG AGT CCA TTG CCA.

Investigation of Confounding Factors

Using information collected from the guardian, we investigated 29 possible confounding factors of the relationship between excessive fluoride and thyroid abnormalities (goiter, thyroid nodules, thyroid disease, and thyroid antibody positive): (1) the children's basic information (age, sex, BMI, BSA, urinary iodine); (2) the children's living habits and physical conditions (exposure time of electronic instrument, eating seafood frequently, eating pickled food frequently, passive smoking, wash or peel vegetables and fruits, physical activity, pressure, anxiety, anger, psychological trauma, having a cold, and radiographic examination of the head and

neck); (3) the children's living environment (noise in surroundings, factory within 30 m of residence, farm/orchard within 30 m of residence); (4) family status (thyroid disease in relatives, father's education, mother's education, father having a stable job, mother having a stable job); and (5) conditions during pregnancy (mother's alcohol drinking during pregnancy, mother's smoking during pregnancy, mother's passive smoking during pregnancy, birth abnormal). The above confounding factors were determined according to the published articles [10, 20, 21] and our understanding of thyroid diseases.

Statistical Methods

In the present study, the children's heights were normally distributed and were described as the mean ± standard deviation (SD). The comparison of height between the two groups was performed using t-test. Other continuous indicators of the children were non-normally distributed variables and were shown as median (P_{25}-P_{75}). The difference between groups was compared using nonparametric test (the Mann-Whitney U test was used to compare 2 groups, and the Kruskal-wallis H test was used to compare 3 or more groups). The categorical variables were described as frequency [proportion (%)][f(%)].The difference in the distribution of thyroid diseases, thyroid antibodies, and nodules was determined using chi-square test or Fisher's exact test. A non-parametric test was used to test differences in the distribution of the FT_3, FT_4, and TSH levels between different groups, because the indicator variables (low, medium and high) were ordered.

Multivariate logistic analysis was used to detect the effects of excessive fluoride on the thyroid health and was divided into 4 models: model 1 was a crude model, and dental fluorosis was the only independent variable; in model 2, the independent variable was 29 confounding factors respectively; model 3 was an adjusted model, and the independent variables were dental fluorosis and positive factors ($P < 0.05$) from model 2; in model 4, after comprehensively considering the sample size, model 3 was build in the subgroups of TSHR rs2268458 = CC+CT and TT or the subgroups of PTPN22 rs3765598 = CC and CT+TT.

Logistic analysis was used to establish a multiplicative interaction model between environmental factors (excessive fluoride) and the SNPs of genes. We used the Andersson method [22] to establish an additive interaction model between excessive fluoride and the SNPs of genes, and then calculated the relative excess risk (RERI), attribute proportion (AP), and synergy index (S) of the interaction.

In all the analyses, the statistical significance level α was set as 0.05 for a two-sided test. All the statistical analyses were performed using SAS university edition (SAS Institute, Cary, North Carolina, USA) and SPSS 24.0 (IBM, Chicago, IL, USA).

Results

Basic Characteristics of the Children

The study comprised 272 normal children aged 9.84 (9.09–10.32) years, and 141 children with dental fluorosis aged 9.35 (8.64–10.00) years. Because of the significant difference in age ($Z = 4.03$, $P < 0.001$), we divided the age groups into < 9, 9–10, and ≥ 10 years to evaluate the characteristics of the children. Table 1 shows no significant difference in the urinary iodine concentration in all the groups (all $P > 0.05$), while the urinary fluoride concentration of children with dental fluorosis was higher than that of normal children (all $P < 0.001$). The weight and BSA of children in the 9–10 years old group were higher than those of normal children (all $P < 0.05$).

Comparison of Thyroid Hormone and Volume in Different Children

Table 2 shows that the FT_3 level of children with dental fluorosis in the 9–10 years old group and the overall participants was increased ($P < 0.05$). In the 9–10, ≥ 10 years groups, and the overall participants, the FT_4 and TGAb levels and thyroid volume decreased (all $P < 0.05$), while the TPOAb levels increased (all $P < 0.05$) in children with dental fluorosis.

Comparison of Thyroid Hormone Abnormalities, Thyroid Diseases and Nodules in Different Children

Table 3 shows that compared with normal children, the proportion of FT_4 higher-than-normal was lower ($P < 0.05$), the positive rate of TPOAb was higher ($P < 0.05$), and the single positive ratio of thyroid antibody was significantly increased ($P < 0.05$) in children with dental fluorosis. Additionally, the ratio of goiter (BSA) and goiter (age or BSA) in children with dental fluorosis was significantly reduced ($P < 0.001$). Therefore, we chose thyroid antibody single positive and goiter (age or BSA) as dependent variables to evaluate their relationship with excessive fluoride.

Distributions of Thyroid Antibody Single Positive and Goiter (Age or BSA) Among the Other Characteristics of the Children

We analyzed the relationship between the 29 confounding factors and thyroid antibody single positive and goiter (age or BSA). From Table s1, it can be seen that the ratio of thyroid antibody single positive in children with father having a stable job was significantly lower ($P < 0.05$). A statistically significant difference was found in the number of goiters (age or BSA) in different age groups ($P < 0.05$). Children who often ate seafood had a lower rate of goiter (age or BSA) ($P < 0.05$),

Table 1 Comparison of characteristics of children in different groups

	Age								Total		
	< 9 years			9~10 years			≥ 10 years				
	Normal (57)	Dental fluorosis (51)	*P*	Normal (106)	Dental fluorosis (54)	*P*	Normal (109)	Dental fluorosis (36)	*P*	Normal (272)	Dental fluorosis (141)	*P*
Height (cm)	134.35±7.01	134.14±6.87	0.873	140.38±7.67	143.39±5.5	0.011	147.45±6.87	147.6±8.49	0.916	141.95±8.78	141.12±8.77	0.362
Weight (kg)	29.80 (25.90~35.10)	28.00 (24.50~35.30)	0.440	32.55 (28.00~42.00)	35.50 (30.60~44.70)	0.040	39.00 (33.00~48.50)	37.25 (32.55~59.25)	0.704	35.00 (29.00~43.00)	34.00 (28.80~44.00)	0.580
BMI (kg/m^2)	16.15 (14.7~17.86)	15.83 (14.3~18.48)	0.455	17.05 (14.92~20.83)	17.36 (15.61~21.63)	0.276	18.23 (15.82~21.02)	18.73 (15.83~23.91)	0.411	17.36 (15.27~20.52)	16.86 (15.2~20.83)	0.862
Surface area (m^2)	1.07 (0.97~1.15)	1.04 (0.95~1.18)	0.520	1.16 (1.04~1.28)	1.22 (1.12~1.33)	0.015	1.27 (1.17~1.44)	1.21 (1.16~1.58)	0.810	1.18 (1.07~1.32)	1.17 (1.06~1.32)	0.545
UI (μg/L)	171.30 (107.30~247.60)	159.10 (113.80~269.90)	0.756	165.45 (98.40~255.4)	154.95 (94.60~253.30)	0.549	166.90 (113.00~262.50)	182.60 (102.85~257.80)	0.891	167.00 (106.55~257.30)	159.20 (106.50~255.90)	0.734
UF (mg/L)	0.84 (0.58~1.66)	1.82 (0.91~3.78)	<0.001	0.75 (0.5~1.16)	1.37 (0.84~1.97)	<0.001	0.78 (0.54~1.07)	1.35 (0.74~1.62)	<0.001	0.78 (0.54~1.21)	1.46 (0.82~2.41)	<0.001
Sex												
Boys	24 (42.11)	23 (45.10)	0.754	46 (43.40)	23 (42.59)	0.923	63 (57.80)	19 (52.78)	0.598	133 (48.90)	65 (46.10)	0.589
Girls	33 (57.89)	28 (54.90)		60 (56.60)	31 (57.41)		46 (42.20)	17 (47.22)		139 (57.10)	76 (53.90)	

UI, urinary iodine; *UF*, urinary fluorine

Height is described as mean ± SD; Sex is described as f(%); others are described as median (P_{25}-P_{75})

Table 2 Comparison of thyroid hormone, antibodies, and volume in different children

	Age								Total		
	< 9 years			9~10 years			≥ 10 years				
	Normal (57)	Dental fluorosis (51)	*P*	Normal (106)	Dental fluorosis (54)	*P*	Normal (109)	Dental fluorosis (36)	*P*	Normal (272)	Dental fluorosis (141)	*P*
FT_3 (pmol/L)	6.54 (6.17~6.96)	6.45 (6.12~7.02)	0.902	6.55 (6.1~6.88)	6.95 (6.4~7.35)	0.002	6.38 (6.01~6.82)	6.61 (6.3~7.02)	0.121	6.49 (6.08~6.93)	6.59 (6.23~7.15)	0.007
FT_4 (pmol/L)	17.72 (16.45~19.2)	17.7 (16.56~19.35)	0.868	18.68 (17.28~19.78)	17.08 (15.95~18.33)	<0.001	18.35 (16.76~19.96)	17.04 (15.85~18.56)	0.013	18.39 (16.94~19.77)	17.3 (16.07~18.6)	<0.001
TSH (uIU/ml)	3.1 (2.55~4.31)	2.74 (2.11~4.37)	0.160	3.09 (2.23~4.03)	2.82 (2.12~4.11)	0.384	2.75 (2.15~3.57)	2.8 (2.18~4.22)	0.488	2.9 (2.24~3.82)	2.75 (2.13~4.21)	0.584
TGAb (IU/mL)	0 (0~10.61)	0 (0~10.55)	0.969	11.53 (10.16~13.2)	0 (0~0)	<0.001	11.18 (10.26~12.3)	0 (0~11.45)	0.002	10.82 (0~12.47)	0 (0~10.55)	<0.001
TPOAb (IU/mL)	9.12 (8.18~10.8)	9.95 (7.89~12.89)	0.210	8.34 (7.05~10.02)	10.43 (8.43~12.42)	<0.001	7.81 (6.7~9.57)	8.85 (7.47~11.24)	0.015	8.34 (6.95~10.02)	9.83 (8.04~12.03)	<0.001
Thyroid volume (ml)	2.14 (1.74~2.56)	2.17 (1.77~2.76)	0.510	3.63 (2.97~4.49)	2.53 (2.17~3.32)	<0.001	4.1 (3.48~4.82)	3.11 (2.41~3.83)	<0.001	3.63 (2.66~4.44)	2.52 (2.02~3.27)	<0.001

Variables are described as median (P_{25}-P_{75})

Table 3 Comparison of thyroid hormone abnormality, thyroid diseases, thyroid antibodies, and nodules in different children

Groups	N	FT_3[a]			FT_4[a]			TSH[a]			TGAb		TPOAb	
		Low	Normal	High	Low	Normal	High	Low	Normal	High	Normal	Positive	Normal	Positive
Normal	272	4 (1.47)	264 (97.06)	4 (1.47)	3 (1.10)	199 (73.16)	70 (25.74)	7 (2.57)	241 (88.60)	24 (8.82)	264 (97.06)	8 (2.94)	266 (97.79)	6 (2.21)
Dental fluorosis	141	3 (2.13)	135 (95.74)	3 (2.13)	1 (0.71)	121 (85.82)	19 (13.48)	5 (3.55)	123 (87.23)	13 (9.22)	135 (95.74)	6 (4.26)	131 (92.91)	10 (7.09)
P		1.000			0.007			0.882			0. 492		0.015	
Groups	N	Ohyper		Shyper		Ohypo		Shypo		Thyroid antibodies				
										Double positive		Single positive		
		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	
Normal	272	272 (100)	0 (0.00)	272 (100)	0 (0.00)	272 (100)	0 (0.00)	256 (94.12)	16 (5.88)	270 (99.26)	2 (0.74)	260 (95.59)	12 (4.41)	
Dental fluorosis	141	141 (100)	0 (0.00)	141 (100)	0 (0.00)	141 (100)	0 (0.00)	128 (90.78)	13 (9.22)	139 (98.58)	2 (1.42)	127 (90.07)	14 (9.93)	
P		–		–		–		0.208		0.887		0.029		
Groups	N	Goiter (age)		Goiter (BSA)		Goiter (age and BSA)		Goiter (age or BSA)		Thyroid nodules				
		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes			
Normal	272	258 (94.85)	14 (5.15)	241 (88.60)	31 (11.40)	266 (97.79)	6 (2.21)	223 (85.66)	39 (14.34)	197 (72.43)	75 (27.57)			
Dental fluorosis	141	139 (98.58)	2 (1.42)	139 (98.58)	2 (1.42)	141 (100.00)	0 (0.00)	137 (97.16)	4 (2.84)	109 (77.30)	32 (22.70)			
P		0.063		< 0.001		0.179		< 0.001		0.283				

Variables are described as f(%)

[a] Nonparametric test was used. Chi square, correction chi square, or Fisher's exact probability method was used in other indicators

Table 4 Distributions of antibodies single positive, goiter (age or BSA) in children with different genotypes of TSHR rs2268458 and PTPN22 rs3765598

Gene	Polymorphism	Antibodies single positive			Goiter (age or BSA)		
		No	Yes	*P*	No	Yes	*P*
TSHR rs2268458	CC	31 (96.88)	1 (3.13)	0.735	29 (90.63)	3 (9.38)	0.052
	CT	146 (93.59)	10 (6.41)		147 (94.23)	9 (5.77)	
	TT	208 (93.27)	15 (6.73)		193 (86.55)	30 (13.45)	
	CC	31 (96.88)	1 (3.13)	0.709	29 (90.63)	3 (9.38)	1.000
	CT+TT	354 (93.4)	25 (6.6)		340 (89.71)	39 (10.29)	
	CC+CT	177 (94.15)	11 (5.85)	0.717	176 (93.62)	12 (6.38)	0.018
	TT	208 (93.27)	15 (6.73)		193 (86.55)	30 (13.45)	
PTPN22 rs3765598	CC	241 (93.05)	18 (6.95)	0.782	234 (90.35)	25 (9.65)	0.131
	CT	125 (94.7)	7 (5.3)		114 (86.36)	18 (13.64)	
	TT	20 (95.24)	1 (4.76)		21 (100)	0 (0)	
	CC	241 (93.05)	18 (6.95)	0.488	234 (90.35)	25 (9.65)	0.498
	CT+TT	145 (94.77)	8 (5.23)		135 (88.24)	18 (11.76)	
	CC+CT	366 (93.61)	25 (6.39)	1.000	348 (89)	43 (11)	0.149
	TT	20 (95.24)	1 (4.76)		21 (100)	0 (0)	

Variables are described as f(%)

and those with farm/orchard within 30 m of residence also had a higher rate of goiter (age or BSA) ($P < 0.05$).

Relationship Between the TSHR (rs2268458) and PTPN22 (rs3765598) SNPs and Thyroid Antibody Single Positive and Goiter (Age or BSA)

The Hardy-Weinberg genetic balance test was performed on the TSHR rs2268458 and PTPN22 rs3765598 polymorphisms. The SNPs of these two genes met the Hardy-Weinberg genetic balance, and the samples were representative (Table s2).

According to the subtypes of the TSHR (rs2268458) and PTPN22 (rs3765598) polymorphisms, we grouped children into three models: (1) CC *vs*. CT *vs*. TT; (2) CC *vs.* CT+TT; and (3) CC+CT *vs.* TT. The distribution differences of children with thyroid antibody single positive or goiter (age or BSA) in the different models were then evaluated. From Table 4, we found that only children with the TSHR rs2268458 polymorphism = TT had more goiters (age or BSA) than those with the CC+CT genotype ($P < 0.05$).

Relationship Between Dental Fluorosis and Thyroid Antibody Single Positive in Different Genotypes

Multivariate logistic analysis was used to analyze the relationship between fluoride excess and thyroid antibody single positive in the overall participants and children carrying two genotypes of TSHR rs2268458 (CC+CT and TT) or two genotypes of PTPN22 rs3765598 (CC and CT+TT) respectively. As shown in Table 5, in the overall participants, both crude and adjusted models found that dental fluorosis was positively associated with the occurrence of thyroid antibody single positive ($P = 0.033$, *OR* (*95%CI*) = 2.39 (1.07–5.31); $P = 0.039$, *OR* (*95%CI*) = 2.35 (1.05–5.27)). It can also be seen from Table 5 that in children carrying TSHR rs2268458

Table 5 Relationship between dental fluorosis and thyroid antibodies single positive in children with different genotypes

		N	Crude model		Adjusted model	
			P	*OR (95%CI)*	*P*	*OR (95%CI)*
	Overall	413	0.033	2.39 (1.07~5.31)	0.039	2.35 (1.05~5.27)
TSHR rs2268458	CC+CT	188	NA	NA	0.036	4.34 (1.10~17.10)
	TT	223	NA	NA	0.336	1.66 (0.56~4.95)
PTPN22 rs3765598	CC	259	NA	NA	0.002	5.40 (1.85~15.81)
	CT+TT	153	NA	NA	0.178	0.23(0.03~1.97)

Adjusted factor:father having a stable job

polymorphism =CC+CT, dental fluorosis was positively associated with the occurrence of thyroid antibody single positive ($P = 0.036$, *OR* (*95%CI*) = 4.34 (1.10–17.10)), and in children carrying PTPN22 rs3765598 polymorphism = CC, dental fluorosis was positively associated with the occurrence of thyroid antibody single positive ($P = 0.002$, *OR* (*95%CI*) = 5.40 (1.85–15.81)).

Relationship Between Dental Fluorosis and Goiter (Age or BSA) in Different Genotypes

Multivariate logistic analysis was also used to analyze the relationship between dental fluorosis and goiter (age or BSA) in the overall participants and children carrying two genotypes of TSHR rs2268458 (CC+CT and TT) or two genotypes of PTPN22 rs3765598 (CC and CT+TT). Table 6 shows that in the overall participants, both crude and adjusted model found that dental fluorosis was inversely associated with occurrence of goiter (age or BSA) ($P = 0.001$, *OR* (*95%CI*) = 0.17 (0.06–0.50); $P = 0.003$, *OR* (*95%CI*) = 0.20 (0.07–0.58)). We also found that in children carrying TSHR rs2268458 polymorphism = TT, dental fluorosis was inversely associated with occurrence of goiter (age or BSA) ($P = 0.018$, *OR* (*95%CI*) = 0.09 (0.01–0.67)). In children carrying PTPN22 rs3765598 CC type, dental fluorosis was inversely associated with occurrence of goiter (age or BSA) ($P = 0.013$, *OR* (*95%CI*) = 0.15 (0.03–0.67)).

Multiplicative and Additive Interactions of Fluoride Exposure and SNP of TSHR (rs2268458) or PTPN22 (rs3765598) on Thyroid Antibody Single Positive or Goiter (Age or BSA)

As Table 7 shown, logistics analysis found that in the multiplicative model, only the interaction between the fluoride exposure (dental fluorosis) level and SNP of PTPN (rs3765598) on thyroid antibody single positive was found ($P = 0.012$, *OR*(*95%CI*) = 0.05 (< 0.01–0.51)). It can also be seen from Table 7 that in the additive model, only the S value (*S*(*95%CI*) = 0.36 (0.13–0.95)) was consistent with the additive interaction between the fluoride exposure (dental fluorosis) level and the SNP of TSHR (rs2268458) for goiter (age or BSA), but the RERI value (*RERI* (95%CI) = − 8.18 (− 27.76–11.40)), AP value (*AP* (*95%CI*) = − 1.49 (− 3.56–0.59)) and Fig. s1 do not support this finding. Therefore, we estimated no additive interaction occurred. No other multiplicative or additive interactions were found between the fluorine exposure level and gene polymorphisms.

Discussion

Fluorine is the thirteenth most abundant element; however, excessive intake will cause damage to multiple systems and organs, not only affecting the bone system, but also damaging the nerves and reproductive systems [4]. The body can take in excessive fluoride by drinking tea containing fluorine, eating fluorine-contaminated salt, or burning coal rich in fluorine, and the most important way is to drink high-fluoride groundwater. Three main high-fluoride groundwater belts exist worldwide: Syria, Jordan, Egypt, Libya, Algeria, Sudan and Kenya; Turkey, Iraq, Iran, Afghanistan, India, Thailand and China; America, and Japan [6]. Therefore, fluorosis is a global public health concern. Many studies have investigated the effects of excessive fluoride on health. Some have used external environmental exposure indexes such as water fluoride [10]. Others have used biomarkers, including fluoride in the teeth, bone surface, nails, plasma, urine, emulsion, and saliva. However, these markers have drawbacks. For example, nails can only reflect fluoride exposure in the past 3–4 months, and are not as good as urinary fluoride to represent of the fluoride concentration in the external environment [23]. The bone surface, blood, emulsion, and saliva fluoride are rarely used in research because of the difficulty in sample collection or insufficient evidence to show their effectiveness [24]. Urinary fluoride and dental fluorosis are the two most widely used indicators. Urinary fluoride is an indicator that reflects short-term fluoride exposure and cannot represent the individual level [24]. Dental fluorosis reflects the early fluorine exposure level because the body is exposed to excessive fluoride during

Table 6 Relationship between dental fluorosis and goiter(age or BSA) in children with different genotypes

		N	Crude model		Adjusted model	
			P	*OR(95%CI)*	*P*	*OR(95%CI)*
	Overall	413	0.001	0.17 (0.06~0.50)	0.003	0.20 (0.07~0.58)
TSHR rs2268458	CC+CT	188	NA	NA	0.317	0.50 (0.13~1.95)
	TT	223	NA	NA	0.018	0.09 (0.01~0.67)
PTPN22 rs3765598	CC	259	NA	NA	0.013	0.15 (0.03~0.67)
	CT+TT	153	NA	NA	0.131	0.30 (0.06~1.43)

Adjusted factors:age, eating seafood frequently, farm/orchard within 30 m of residence

Table 7 The interaction between dental fluorosis and TSHR rs2268458 or PTPN22 rs3765598 in the effects of antibodies single position and goiter (age or BSA)

			Multiplicative model		Additive model		
		P	*β*	*OR (95%CI)*	*RERI (95%CI)*	*AP (95%CI)*	*S (95%CI)*
Antibodies single positive[a]	Fluoride*rs2268458	0.281	−0.964	0.38 (0.07~2.20)	− 1.77 (− 7.65~4.12)	− 0.45 (− 2.01-1.12)	0.63 (0.16-2.49)
	Fluoride*rs3765598	0.012	− 3.078	0.05 (< 0.01~0.51)	− 6.28 (− 13.95-1.38)	− 10.66 (− 34.92-13.60)	− 0.07 (NA~NA)
Goiter (age or BSA)[b]	Fluoride *rs2268458	0.156	− 1.771	0.17 (0.02~1.96)	− 8.18 (− 27.76~11.40)	− 1.49 (− 3.56~0.59)	0.36 (0.13~0.95)
	Fluoride *rs3765598	0.458	− 0.812	0.44 (0.05~3.79)	− 0.37 (− 7.14~6.39)	− 0.05 (− 0.87~0.78)	0.95 (0.39~2.33)

[a] Adjusted factor: father having a stable job

[b] Adjusted factors: age, eating seafood frequently, farm/orchard within 30 m of residence

enamel formation. When selecting the subjects in this study, children were selected based on fluoride concentration in the water of the residence, and grouped by the levels of dental fluorosis with urinary fluoride as a supplement. The present study found that the urinary fluoride level of children with dental fluorosis was significantly higher than that of children in the normal group. Therefore, we believe that our method is more representative of children's fluoride exposure level.

Presently, studies on the effects of excessive fluoride on thyroid function and structure are inconsistent. Abdelaleem et al. [7] administered drinking water containing 15 mg/kg of NaF to Sprague Dawley albino rats for 2 weeks through a gastric tube, and found that the serum T_3 and T_4 decreased, the TSH level increased, thyroid follicular cells were degraded, and the number of follicles was decreased. Liu et al. [9] exposed Wistar rats to water with excessive fluoride (up to 20 mg/L of NaF), and found that the serum T_4 and FT_4 levels increased after 2 months and the TSH was decreased after 8 months, with a decrease in the volume and number of thyroid follicles. Additionally, Wang et al. [10] investigated the relationship among the water fluoride, urinary fluoride, and thyroid hormone levels in 571 children in areas with 0.2 to 3.9 mg/L of water fluoride and found that elevated water and urinary fluoride levels increased the TSH level and the urinary fluoride level decreased the T_4 and FT_4 levels. However, Barberio et al. [11] investigated the relationship between the water fluoride, urinary fluoride, and thyroid hormones levels and disease in more than 5000 people aged 3–79 years in the drinking water fluoridation area of Canada; they found that the urine fluoride, water fluoride, and TSH levels and hypothyroidism were not related. Our study found that the FT_4 and TGAb levels and thyroid volume of children exposed to excessive fluoride were significantly reduced, and the TPOAb levels was increased. Several potential mechanisms exit: (1) fluorine is an analogue of iodine, which is considered to have higher biological activity than iodine, affecting the uptake of iodine by the thyroid, hindering the function of the thyroid, and disrupting thyroid hormones production [25]; (2) fluorine is an analog of TSH that more easily binds to the TSH receptor than TSH, thus affecting the stability of thyroid hormones [26, 27]; (3) fluoride can also induce endoplasmic reticulum stress in thyroid cells [9], and affect the secretion of thyroid hormones and some antibodies. Decreased secretion leads to smaller follicles, which explain the fluorine-induced thyroid volume reduction; (4) the decrease in thyroid volume caused by excessive fluoride may also be related to the induction of thyroid cell apoptosis [9].

TSHR is a key factor for maintaining normal thyroid function. It can combine with TSH to activate the thyroid-stimulating hormone receptor/cyclic adenosine monophosphate (TSHR/cAMP) pathway, and promote the normal functioning of sodium iodide symporter (NIS). TSHR SNPs (rs74067403, rs1054708, rs1991517, rs2288496) are related to the levels of thyroid hormone and TGAb in patients with AITD, Hashimoto's thyroiditis, and papillary thyroid cancer [28–30]. However, few studies have investigated the effect of TSHR-SNP-rs2268458 on thyroid health. The polymorphism of this locus may be related to the occurrence and recurrence of Graves' disease (GD) [31, 32]. However, the relationship between this polymorphism and other changes in thyroid structure and function has not been studied. Our study explored the relationship between the TSHR SNP (rs2268458) and thyroid antibody single positive and goiter (age or BSA). We found that the prevalence of goiter (age or BSA) in children with TSHR rs2268458 polymorphism=TT was significantly increased. Studies have shown that PTPN22 gene SNPs exert different effects on thyroid diseases. Alkhateeb et al. [15] found that PTPN22-SNP-rs2476601 of Jordanian Arabs has no relationship with Hashimoto and GD. However, Luo et al. [17] used a meta-analysis to find that among Caucasians, PTPN22-SNP-C1858T may be related to AITD. PTPN-SNP-rs3765598 may be related to systemic lupus erythematosus (SLE) [33]. We found no reports on the relationship between PTPN-SNP-rs3765598 and thyroid disease; thus, we focused on to the relationship between PTPN-SNP-rs3765598 and thyroid antibody single positive and goiter (age and BSA), but found no relationship. Additionally, we focused on the moderating roles

of TSHR-SNP-rs2268458 and PTPN22-SNP-rs3765598 in the effects of excessive fluoride on thyroid antibody single positive and goiter (age or BSA). We found that, in children in TSHR (rs2268458) CC+CT subgroup or PTPN22 (rs3765598) CC group, excessive fluoride was positively associated with the occurrence of thyroid antibody single positive. Furthermore, we found that, in children carrying the TSHR rs2268458 polymorphism =TT or the PTPN22 rs3765598 polymorphism =CC, excessive fluoride was inversely associated with occurrence of goiter (age and BSA), i.e., excessive fluoride exposure may reduce the thyroid volume.

We also used a multiplicative model and an additive model to study the interaction of fluoride exposure level with the TSHR (rs2268458) or PTPN22 (rs3765598) SNP. The interaction is mainly divided into statistical interaction and biological interaction. Using different statistical models, the results of statistical interactions may be inconsistent, and biological interactions are more widely accepted. Some scholars have proposed that the additive model is more accurate when discussing biological interactions; therefore, in etiology epidemiology, the biological interaction of the additive model is more valued [34, 35]. Our study only found that the fluoride exposure levels and SNP of PTPN22 (rs3765598) had a multiplicative interaction when acting on thyroid antibody single positive, but no additive interaction was found, nor was there a multiplicative or additive interaction when acting on goiter (age or BSA). Similarly, no multiplicative or additive effect was found between fluoride exposure and the TSHR SNP (rs2268458) on thyroid antibody single positive and goiter (age or BSA). In summary, no biological interactions were found between the fluoride exposure levels and TSHR-SNP-rs2268458 or PTPN22-SNP-rs3765598 when acting on thyroid antibody single positive and goiter (age or BSA).

The advantage of our study is that 29 confounding factors were fully considered when exploring the relationship between excessive fluoride and thyroid antibody single positive and goiter (age or BSA). To our best knowledge, this study is the first time to explore the role of TSHR-SNP-rs2268458 and PTPN22-SNP-rs3765598 in the effects of excessive fluoride on the thyroid. However, this study also has limitations. To ensure the sample size of the logistic model, the genotypes of TSHR (rs2268458) and PTPN22 (rs3765598) were only grouped according to our method, because the distribution of different genotypes in the population was uneven.

Conclusion

Children over 9 years old with dental fluorosis have lower FT_4 and TGAb levels and thyroid volume and higher TPOAb levels. For children with the TSHR (rs2268458) CC+CT genotype or PTPN22 (rs3765598) CC genotype, excessive fluoride exposure was positively associated with the occurrence of antibody single positive. Excessive fluoride exposure in children with TSHR (rs2268458) TT genotype or PTPN22 (rs3765598) CC genotype was inversely associated with occurrence of goiter (age or BSA). No biological interaction was found between fluoride exposure and SNP of TSHR (rs2268458) or PTPN22 (rs3765598).

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Data Availability Data and material are available on request from the correspondence author or first author.

Code Availability Not applicable.

Declarations

Ethics Approval The study was approved by the ethics committee of Tianjin Centers for Disease Control and prevention (approval No.: TJCDC199). All children are approved by their guardians and our study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent to Participate All participants have consented to participate to this study.

Consent for Publication The participants have consented to the submission of the manuscript to the journal.

Conflict of Interest The authors declare no competing interests.

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