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**Subclinical thyroid disease and single nucleotide polymorphisms in reproductive-age women in areas of Shanxi Province, China, where iodine exposure is excessive**

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**Running title:** SNPs in subclinical thyroid disease

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## ABSTRACT

**Background and Objectives:** When iodine intake is in excess, a susceptible population that has a genetic predisposition will have an increased risk of hypothyroidism or autoimmune thyroiditis. This study evaluated the vulnerability to iodine excess and subclinical thyroid disease through screening of single nucleotide polymorphisms (SNPs) in reproductive-age women to provide evidence to be used for the prevention of subclinical thyroid disease.

**Methods and Study Design:** In Shanxi province, four areas where a range of iodine exposures from low to high were chosen. In each region, 60 women were anticipated to enrol, including 20 pregnant women, 20 lactating women, and 20 non-pregnant, non-lactating women. Genotyping was performed using whole-blood samples, and the genotypes of 21 SNPs were determined and compared among areas with different water iodine and between controls and patients with subclinical thyroid disease.

**Results:** In total, 241 participants were enrolled. Among the 21 candidate SNPs, no difference was found among areas with various water iodine, whereas, *TG* (rs2252696), *TSHR* (rs4903957), *CTLA-4* (rs231775), *CAPZB* (rs1472565), *PDE4D* (rs27178), and *HLA* (rs2517532) were significantly associated with various subclinical thyroid diseases; in particular, the *PDE4D* (rs27178) ad hoc TT allele was associated with all examined subclinical thyroid diseases.

**Conclusions:** Vulnerability to subclinical thyroid diseases is influenced by the presence of gene polymorphisms. There is a need for screening of suspected genes to effectively prevent and reduce the occurrence of thyroid diseases. People with the TT allele in *PDE4D* (rs27178) should be made aware of an increased risk of subclinical thyroid disease.

**Key Words:** iodine, subclinical thyroid disease, susceptible population, SNP, screening

## INTRODUCTION

The thyroid is an endocrine gland that produces thyroid hormone. The thyroid hormone regulates many activities in the body, including the speed at which the body burns calories and the rate at which the heart beats. Diseases of the thyroid cause it to produce either too much or too little of the hormone. Women are more likely than men to have thyroid diseases, especially right after pregnancy.<sup>1</sup> According to the World Health Organization's manual on iodine deficiency disorders, when the median urinary iodine of children or adults reaches 200–299 µg/L, susceptible populations with a genetic predisposition will have an increased risk of hypothyroidism or autoimmune thyroiditis. In addition to overt thyroid disease, there are subclinical thyroid diseases that have no obvious clinical symptoms and can only be

diagnosed based on laboratory tests. Subclinical thyroid diseases include subclinical hyperthyroidism, subclinical hypothyroidism, hypothyroxinaemia, and diseases caused by the presence of positive thyroid antibody.<sup>2</sup> Hereditary factors—mainly the regulation of genetic factors—play a role in thyroid diseases.<sup>3</sup> Single nucleotide polymorphisms (SNPs) are involved in both thyroid functioning and thyroid autoimmune disorders.<sup>4,5</sup> Some genes and their loci have an association with thyroid-stimulating hormone (TSH) concentrations and can influence the development of hypothyroidism, including *FOXE1* (forkhead box protein E1),<sup>6</sup> *SH2B3* (Src homology 2-B3), *PTPN22* (protein tyrosine phosphatase non-receptor 22), *CAPZB* (capping protein, actin filament, muscle Z-line, beta), *PDE8B* (phosphodiesterase 8B), *CTLA-4* (cytotoxic lymphocyte-associated antigen-4), *HLA* (human leucocyte antigen) class II, *THRB* (thyroid hormone receptor, beta), *TG* (thyroid globulin), *POU1F1* (POU class 1 homeobox 1), *PDE4D* (phosphodiesterase 4D), *TSHR* (thyroid-stimulating hormone receptor) and *GNAQ* (guanine nucleotide-binding protein G (q)).<sup>7</sup> Furthermore, polymorphisms in *PDE8B* may affect the production of T4 and T3 and the regulation the release of TSH by the pituitary gland; hence, *PDE8B* may serve as a candidate target for the regulation of thyroid dysfunction.<sup>8</sup> Common genetic variation in iodothyronine deiodinase type 1 (*DIO1*) alters deiodinase function, resulting in an imbalance in the circulating FT3 to FT4 ratio, both of which affect thyroid function.<sup>9</sup> Other gene loci are involved in immune functioning and play a role in various autoimmune diseases, including Vav-family protein 3 (*VAV3*) and *CTLA-4*.<sup>7</sup>

Individuals with a susceptible genetic background are vulnerable to iodine excess and thyroid disease. Studies have focused on associations between SNPs and thyroid function parameters<sup>7-9</sup> or thyroid diseases, such as autoimmune thyroid disease<sup>10-14</sup> and thyroid cancer<sup>15-17</sup>; few studies have focused on subclinical thyroid disease. In this study, 21 candidate SNPs chosen from previous reports were genotyped to determine the association between subclinical thyroid disease and SNPs in reproductive-age women in areas of excessive iodine exposure from water in Shanxi Province, China.

## **MATERIALS AND METHODS**

### ***Study participants and methods***

In 2011, four regions in Shanxi Province with water iodine content from low to high and a high prevalence of subclinical thyroid disease were chosen. The water iodine level was classified at four different levels, those being 50–99, 100–149, 149–299, and  $\geq 300$   $\mu\text{g/L}$ . In each region, 60 participants were planned to recruit: 20 pregnant women, 20 lactating women, and 20 controls (non-pregnant, non-lactating reproductive-age women); only women were

chosen because the prevalence of thyroid disease is higher in women than in men, especially during pregnancy or lactation. The study protocol was approved by the Human Research Ethics Committee at the Harbin Medical University (approval number HRB2010e005). The research conformed to the provisions of the Declaration of Helsinki, 1995 (as revised in Edinburgh, 2000). Informed consent was obtained from all participants. From each person, a blood sample was collected, and thyroid function parameters (TSH, thyroid stimulating hormone, FT<sub>4</sub>, free thyroxine, FT<sub>3</sub>, free triiodothyronine, TgAb, thyroglobulin antibody, TPOAb, thyroid peroxidase antibody) were determined. TSH, FT<sub>4</sub>, and FT<sub>3</sub> were measured using an electrochemical luminescence immunoassay (Roche, Germany). TgAb and TPOAb were measured using commercial kits (Roche) according to manufacturer's instructions. The reference ranges were as follows: TSH, 0.27–4.20 mIU/L; FT<sub>4</sub>, 12–22 pmol/L; FT<sub>3</sub>, 2.8–7.1 pmol/L; TgAb, 0–115 IU/mL; TPOAb, 0–34 IU/mL. For the purposes of analysis, patients with subclinical thyroid diseases were defined as those having positive thyroid antibody, subclinical hypothyroidism, or hypothyroxinaemia (low T<sub>4</sub> concentration), or any of those three. Additionally, subclinical thyroid disease was diagnosed based on the serological concentration of thyroid functional parameters: positive thyroid antibody, TgAb >115 IU/mL or TPOAb >34 IU/mL; subclinical hypothyroidism, TSH >4.20 mIU/L and FT<sub>4</sub> 12–22 pmol/L; low T<sub>4</sub> concentration, FT<sub>4</sub> <12 pmol/L. The participants were categorised into cases (i.e., patients with subclinical thyroid disease) or controls based on these indices.

### ***DNA extraction***

For all participants, genomic DNA was extracted from peripheral blood cells using the QIAGEN kit (Qiagen, Germany). Selection of SNPs related to serum TSH and FT<sub>4</sub> in this study was based mainly on results from genome-wide association studies of thyroid diseases (in both the English language and Chinese literature).<sup>4,8,18-21</sup> Genetic loci were selected for screening if significant associations existed between polymorphisms of these loci and iodine excess and between SNP and thyroid disease. After cautiously reviewing the references, the following 21 SNPs were included: *FOXE1* (rs925489, rs1443434), *PTPN22* (rs6679677, rs2476601), *HLA* (rs2517532, rs2516049), *PDE8B* (rs4704397, rs7714529, rs10066802), *SH2B3* (rs3184504), *VAV3* (rs4915077), *CAPZB* (rs1472565), *DIO1* (rs2235544), *CTLA-4* (rs231775, rs3087243), *TG* (rs2252696), *TSHR* (rs4903957), *THRB* (rs1505287), *GNAQ* (rs10512065), *POU1F1* (rs1976324), and *PDE4D* (rs27178). Genotyping was performed by the Shanghai Fenglin Medical Laboratory using the Mass ARRAY Analyzer mass

spectroscope (Agena Bioscience), and the SNP genotyping was analysed using Typer 4.0 (Sequenom).

### ***Statistical analysis***

Genotype comparisons for each SNP between areas with different water iodine were and between subclinical thyroid disease cases and controls performed using the SPSS software (version 17.0, SPSS Inc., Chicago, Illinois, USA), with  $p < 0.05$  regarded as statistically significant. Spearman correlation analysis was used to compare non-normally distributed data. The Hardy–Weinberg equilibrium was tested. The difference in frequencies among different areas and between cases and controls was calculated using the chi-squared test. Risk factors were analysed using logistic regression.

## **RESULTS**

The concept chart of this research was offered in Figure 1. In all, 241 women with an average age of  $29.4 \pm 6.22$  years (19–45 years) were evaluated at 21 SNP loci using the mass spectrometry analysis method; their median urinary iodine level was  $336.00 \mu\text{g/L}$  ( $229.38$ – $526.43 \mu\text{g/L}$ ). Genotypes were compared among populations in various water iodine areas, and between cases and controls. The sample size of population in each area and with each subclinical thyroid disease is presented in Table 1; the Hardy–Weinberg equilibrium was tested and results were balanced. Genotype maps of *PDE4D* (rs27178) are presented as a sample of genotypes CC, CT, and TT in Figure 2.

The possible impairment caused by iodine excess to humans was analysed by comparing the SNP genotypes of populations among areas with different levels of iodine in water; for all 21 SNPs, there was no significant difference found among the different areas, which suggests that iodine excess does not influence the genotype.

As shown in Tables 2 and 3, of the 241 participants, 34 were positive for thyroid antibody and 207 served as controls. Among the 21 SNPs, three loci — *CAPZB* (rs1472565), *PDE4D* (rs27178), and *HLA* (rs2517532) — were significantly associated with positive thyroid antibody. For *CAPZB* (rs1472565), when compared with the TT genotype, the CC genotype was more likely to be positive for thyroid antibodies (odds ratio [OR] = 5.12, 95% confidence interval [CI]=1.76–14.87,  $p < 0.01$ ). Similar associations were observed when the dominant model (TT + TC versus CC) and the recessive model (TT versus TC + CC) were employed (dominant: TT + TC vs. CC, OR=2.74, 95% CI=1.14–6.58,  $p = 0.02$ ; recessive: TT vs. TC + CC, OR=3.18, 95% CI=1.36–7.45,  $p < 0.01$ ). In the dominant model, *PDE4D*

(rs27178) appeared to increase the risk of positive thyroid antibody (OR=2.15, 95% CI=1.00–4.64,  $p=0.05$ ); in the recessive model, *HLA* (rs2517532) appeared to reduce the risk of positive thyroid antibody (OR=0.10, 95% CI=0.01–0.77,  $p=0.03$ ).

In the analysis of those with subclinical hypothyroidism, with some being regarded as cases ( $n=38$ ) and others ( $n=203$ ) as controls, out of the 21 SNPs only TT in *PDE4D* (rs27178) was found to be associated with a risk of subclinical hypothyroidism (OR=5.18, 95% CI=1.75–15.28,  $p<0.01$ ). In the recessive model, *PDE4D* (rs27178) was also found to increase the risk of subclinical hypothyroidism (OR=5.75, 95% CI=2.06–16.06,  $p<0.01$ ).

When participants with hypothyroxinaemia were regarded as cases ( $n=21$ ) and others ( $n=220$ ) as controls, it was observed that among the 21 SNPs, the AA genotype in both *TG* (rs2252696) and *TSHR* (rs4903957) increased the risk of hypothyroxinaemia versus the homozygous wild type. In the recessive model, *CTLA-4* (rs231775), *TG* (rs2252696), and *TSHR* (rs4903957) also appeared to increase the risk of hypothyroxinaemia.

When the presence of any of the three subclinical thyroid diseases was considered, there were 45 cases and 196 controls. It was observed that among the 21 SNPs, only the TT genotype in *PDE4D* (rs27178) increased the total risk of subclinical thyroid disease (OR=5.74, 95% CI=1.97–16.77,  $p<0.01$ ). The recessive model also showed a significant association with risk (OR=5.88, 95% CI=2.13–16.24,  $p<0.01$ ).

## DISCUSSION

Studies have found that some thyroid diseases and subclinical thyroid diseases have higher prevalence in populations subject to iodine excess compared with those whose iodine levels are sufficient or deficient, especially hypothyroidism, subclinical hypothyroidism and autoimmune thyroiditis.<sup>22</sup> Similar differences were also found between populations in areas of iodine excess and areas where iodine levels were adequate.<sup>23,24</sup> A study in which a follow-up survey was conducted five years after the baseline survey demonstrated that those populations with high thyroid peroxidase antibody levels and high thyroglobulin antibody levels in areas of iodine excess had a higher incidence of supernormal thyrotropin than those populations in areas where levels of iodine were adequate.<sup>25</sup> Among those clinical or subclinical thyroid diseases, some diseases were found to be influenced by genetic background.<sup>26</sup> One study in Denmark found that thyroid volume was strongly associated with genetic factors.<sup>27</sup> Other studies have reported the effects of genetic factors and environment on autoimmune thyroid disease (AITD).<sup>28-29</sup> In regards to AITD, existing research focuses mainly on gene polymorphisms. The proposed mechanism is that the *HLA* molecule mistakenly activates T

and B lymphocytes, which triggers the occurrence of AITD.<sup>30</sup> Additionally, polymorphisms in *CD40 C/T-1* and *PTPN22 1858T* are associated with a risk of Graves' disease (GD).<sup>31,32</sup> Amino acid variation at the SNP site of the *CTLA-4* gene was closely associated with AITD.<sup>33</sup>

In the present study, the possible impairment caused by iodine excess was evaluated by comparison of the SNP genotypes of 21 loci among populations in areas with different levels of water iodine; however, no difference was found between them, which suggests that environmental sources of iodine may not have much impact on genetic background in humans.

Besides, the influence of genetic factors on subclinical thyroid disease was explored. Close associations between common variants in *CAPZB* (rs1472565) and the presence of hypothyroidism in patients was found,<sup>4</sup> which may be due to the TSH concentration being affected by *CAPZB* (rs1472565). Lisette et al<sup>8</sup> reported that *PDE4D* (rs27178) and TSH concentrations were associated with changes in thyroid function. TSH is the most sensitive indicator of thyroid dysfunction. In the present study, participants carrying the TT genotype of *PDE4D* (rs27178) were more vulnerable, in that they had a 5.18-fold greater risk of subclinical hypothyroidism than those carrying the CC genotype. In the recessive model, carrying the T allele increased the risk of subclinical hypothyroidism, which supported the presence of an association between *CAPZB* (rs1472565), *PDE4D* (rs27178), and hypothyroidism.

In the dominant model, *PDE4D* (rs27178) could increase the risk of the presence of auto-antibodies for thyroid antibody-positive participants. In the recessive model, *HLA* (rs2517532) decreased this risk to 10%. The rs2517532 SNP lies in the HLA class I region and has always been considered to be related to autoimmune diseases. A genome-wide study reported that *HLA* (rs2517532) is associated with thyroid disease;<sup>4</sup> the present study confirms this association, and variation at this gene locus can greatly increase the chances of the occurrence of positive thyroid antibody.

Variation at the *TG* (rs2252696), *CTLA-4* (rs231775) and *TSHR* (rs4903957) loci and at the recessive models was associated with an increased risk of hypothyroxinaemia. According to one study, *TG* (rs2252696) and *TSHR* (rs4903957) were strongly associated with TSH concentrations and thyroid function.<sup>8</sup>

For at least one of these subclinical thyroid diseases, TT lies in *PDE4D* (rs27178) can increase the risk of subclinical thyroid disease. In the recessive model, carrying the T allele increases the risk of subclinical thyroid disease. TSH or thyroid hormone secretion is influenced mainly by genetic polymorphisms. Among the 21 SNP loci, *CTLA-4* (rs231775),

*TG* (rs2252696), *TSHR* (rs4903957), *CAPZB* (rs1472565), *PDE4D* (rs27178), and *HLA* (rs2517532) were the six sites found to be significantly associated with subclinical thyroid disease, and these may therefore increase the susceptibility of the disease. These six loci have been previously reported to be influential genes in subclinical thyroid disease.<sup>4,8</sup> Among them, only *PDE4D* (rs27178) was found to be associated with any subclinical thyroid disease, which is consistent with research by Weiping et al.<sup>34</sup> Only the TT genotype in *PDE4D* (rs27178) is associated with a significant difference between iodine nutrition-vulnerable groups, including the iodine deficiency damage-susceptible group (TSH <1 mIU/L), the vulnerable group (TSH 1 - 2 mIU/L), and the iodine excess damage-susceptible group (TSH  $\geq$ 2 mIU/L).

This was the first study that screened 21 suspicious gene loci in patients with subclinical thyroid disease; these gene loci were previously reported to be associated with various thyroid diseases, such as thyroid cancer or autoimmune thyroiditis. This study included reproductive-age women, including pregnant and lactating women, from areas with iodine excess for evaluation of vulnerability to iodine excess and subclinical thyroid disease through screening of SNPs. However, the sample size was not large enough, which led to large 95% confidence intervals. Further research with a larger sample size is recommended to obtain more certain conclusions.

The incidence of subclinical thyroid disease in reproductive-age women in areas of iodine excess is influenced by a number of gene loci polymorphisms. There is a need to increase the screening of suspected gene loci in vulnerable populations and to effectively prevent or reduce the occurrence of thyroid diseases by taking into account the influence of their genotypes. Attention should be paid to the risk of subclinical thyroid diseases in people with the TT allele of *PDE4D* (rs27178).

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## REFERENCES

1. A fact sheet from the office on women's health. 2015/01/02. [Cited 2017/10/10]. Available from [http://motherjourney.com/uploads/3/5/3/1/35315324/owh\\_restaurants\\_solutions.pdf](http://motherjourney.com/uploads/3/5/3/1/35315324/owh_restaurants_solutions.pdf).
2. Ross DS. Serum thyroid-stimulating hormone measurement for assessment of thyroid function and disease. *Endocrinol Metab Clin North Am*. 2001;30:245-64.
3. Tomer Y. Genetic susceptibility to autoimmune thyroid disease; past, present, and future. *Thyroid*. 2010;20:715-25.
4. Nicholas E, Joyce YT, Amy KK, David AH, Uta F, Joanna LM, Chuong BD. Novel Associations for Hypothyroidism Include Known Autoimmune Risk Loci. *PLoS One*. 2012;7:3442.
5. Deirdre CE, Alia H, Yaron T. Cutting edge: the etiology of autoimmune thyroid diseases. *Clin Rev Allergy Immuno*. 2011;41:190-7.
6. Zhan M, Chen G, Pan CM, Gu ZH, Zhao SX, Liu W et al. Genome-wide association study identifies a novel susceptibility gene for serum TSH levels in Chinese populations. *Hum Mol Genet*. 2014; 23:5505-17.
7. Eriksson N, Tung JY, Kiefer AK, Hinds DA, Francke U, Mountain JL, Do CB. Novel associations for hypothyroidism include known autoimmune risk loci. *PLoS One*. 2012;7:e34442. doi: 10.1371/journal.pone.0034442.
8. Lisette A L, Gianluca U, Graziano C, Braxton DM, Maria GP, Maria GP et al. Phosphodiesterase 8B Gene Variants Are Associated with Serum TSH Levels and Thyroid Function. *Am J Hum Genet*. 2008; 82:1270-80.
9. Panicker V, Cluett C, Shields B, Anna M, Kirstie SP, John RBP et al. A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. *J Clin Endocrinol Metab*. 2008;93:3075-81.
10. Meng S, He ST, Jiang WJ, Xiao L, Li DF, Xu J, Shi XH, Zhang JA. Genetic susceptibility to autoimmune thyroid diseases in a Chinese Han population: role of vitamin D receptor gene polymorphisms. *Ann Endocrinol (Paris)*. 2015;76:684-9. doi: 10.1016/j.ando.2015.01.003.
11. Wang X, Zhu YF, Li DM, Qin Q, Wang Q, Muhali FS, Jiang WJ, Zhang JA. Polymorphisms of ST2-IL18R1-IL18RAP gene cluster: a new risk for autoimmune thyroid diseases. *Int J Immunogenet*. 2016;43:18-24. doi: 10.1111/iji.12240.
12. Yoshie N, Watanabe M, Inoue N, Kawaguchi H, Hidaka Y, Iwatani Y. Association of polymorphisms in the ICOS and ICOSL genes with the pathogenesis of autoimmune thyroid diseases. *Endocr J*. 2016; 63:61-8. doi: 10.1507/endocrj. EJ15-0435.
13. Alkhateeb A, Marzouka NA, Tashtoush R. Variants in PTPN22 and SMOC2 genes and the risk of thyroid disease in the Jordanian Arab population. *Endocrine*. 2013;44:702-9. doi: 10.1007/s12020-013-9908-z.

14. Barkia Beradhi S, Flesch BK, Hansen MP, Matheis N, Kahaly GJ. HLA class II differentiates between thyroid and polyglandular autoimmunity. *Horm Metab Res.* 2016;48:232-7. doi: 10.1055/s-0035-1559622.
15. Figlioli G, Elisei R, Romei C, Melaiu O, Cipollini M, Bambi F et al. A comprehensive meta-analysis of case-control association studies to evaluate polymorphisms associated with the risk of differentiated thyroid carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2016;25:700-13.
16. Pellé L, Cipollini M, Tremmel R, Romei C, Figlioli G, Gemignani F et al. Association between CYP2E1 polymorphisms and risk of differentiated thyroid carcinoma. *Arch Toxicol.* 2016;12:3099-109.
17. Somuncu E, Karatas A, Ferahman S, Saygili N, Yilmaz E, Ozturk O, Kapan M. The investigation of foxe1 variations in papillary thyroid carcinoma. *Int J Clin Exp Pathol.* 2015;8:13458-64.
18. Marco M, Wendy MD, Michael V. A large-scale association analysis of 68 thyroid hormone pathway genes with serum TSH and FT4 levels. *Eur J Endocrinol.* 2011;164:781-8.
19. Dorota PL, Ewa S, Daria D. CTLA-4 gene polymorphisms and their influence on predisposition to autoimmune thyroid diseases(Graves' disease and Hashimoto's thyroiditis). *Arch Med Sci.* 2012;8:415-21.
20. Naoya I, Mikio W, Hiroya Y. Associations between autoimmune thyroid disease prognosis and functional polymorphisms of susceptibility genes, CTLA4, PTPN22, CD40, FCRL3, and ZFAT, previously revealed in genomewide association studies. *J Clin Immunol.* 2012;32:1243-52.
21. Ming Z, Shuangxia Z, Zhaohui G, Guo CC, Song ZY, Song HD. Association analysis of PDE8B gene polymorphisms with the susceptibility to hypothyroidism in Chinese Han population. *Natl Med J China.* 2012;92:801-5.
22. Du Y, Gao YH, Meng FG, Liu SJ, Fan ZP, Wu JH, Sun DJ. Iodine deficiency and excess coexist in china and induce thyroid dysfunction and disease: a cross-sectional study. *PLoS One.* 2014;9:e111937.
23. Wang D, Du Y, LIU L, Meng FG, Jia QZ, Zhang XY, Wen Da, Jiang P, Shen HM. Study on iodine nutrition status of pregnant women in different water iodine regions and their influencing factors. *J Chin Endemiol Prev.* 2016;1:1-6
24. Liu L, Wang D, Liu P, Meng FG, Wen D, Jia QZ, Liu J, Zhang XY, Jiang P, Shen HM. The relationship between iodine nutrition and thyroid disease in lactating women with different iodine intakes. *BJN.* 2015;114:1487-95.
25. Teng W, Shan Z, Teng X, Guan HX, Li YS, Teng D et al. Effect of iodine intake on thyroid diseases in China. *N Eng J Med.* 2006;26:2783-93.
26. Guarneri F, Benvenega S. Environmental factors and genetic background that interact to cause autoimmune thyroid disease. *Curr Opin Endocrinol Diabetes Obes.* 2007;14:398-409.
27. Hansen PS, Brix TH, Bennedboek FN, Steen JB, Kirsten OK, Laszlo H. Genetic and environmental causes of individual differences in thyroid size: a study of Healthy Danish Twins. *J Clin Endocrinol Metab.* 2004;89:2071-7.

28. Prummel MF, Strieder T, Wiersinga WM. The environment and autoimmune thyroid diseases. *Eu J Endocrinol*. 2004;150:605-18.
29. Marrack P, Kappler J, Kotzin BL. Autoimmune disease: why and where it occurs. *Nat Med*. 2001;7:899-905.
30. Chen XH, Mei YZ, He B, Li HL, Wang X, Hu R, Li L, Ding ZG. General and specific genetic polymorphism of cytokines-related gene in AITD. *Mediators Inflamm*. 2017;1:1-8.
31. Yang J, Qin Q, Yan N, Zhu YF, Li C, Yang XJ, Wang X, Pandey M, Hou P, Zhang JA. CD40 C/T-1 and CTLA-4 A/G(49) SNPs are associated with autoimmune thyroid diseases in the Chinese population. *Endocrin*. 2012;41:111-5.
32. Velaga MR, Wilson V, Jennings CE, Own CJ, Herington S, Donaldson PT et al. The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J Clin Endocrin Metab*. 2004;11:5862.
33. Ban Y, Greenberg DA, Concepcion E, Skrabanek L, Villanueva R, Tomer Y. Amino acid substitutions in the thyroglobulin gene are associated with susceptibility to human and murine autoimmune thyroid disease. *Proc Natl Acad Sci USA*. 2003;25:15119-24.
34. Weiping T, Xiaochun T. Progress iodine and thyroid disease. *Chin J Intern Med*. 2006;26:1569-73.

**Table 1.** Sample size of genotype test

Groups	Sample size
Water iodine	
50-99 µg/L	61
100-149 µg/L	60
150-299 µg/L	61
>300 µg/L	59
Fertility status	
Pregnant women	81
Lactating women	80
Non-pregnant, non-lactating women	80
Subclinical thyroid disease	
Thyroid antibody positive	
Case	34
Control	207
Subclinical hypothyroidism	
Case	38
Control	203
Low T4 concentration	
Case	21
Control	220
Any subclinical thyroid disease	
Case	45
Control	196
<b>Total</b>	<b>241</b>

†Japanese, Thai, Korean, Philippine.

**Table 2.** Association between single nucleotide polymorphisms and water iodine levels

SNP	Genotype	Water iodine ( $\mu\text{g/L}$ , %)				$X^2$	$p$	
		50-99	100-149	150-299	>300			
FOXE1	rs925489	TT	52 (85.25)	51 (85.00)	49 (80.33)	48 (81.36)	2.41	0.88
		CT	9 (14.75)	8 (13.33)	11 (18.03)	10 (16.95)		
		CC	0 (0.00)	1 (1.67)	1 (1.64)	1 (1.69)		
	rs1443434	TT	47 (77.05)	40 (66.67)	40 (65.57)	46 (77.97)	11.81	0.07
		GT	10 (16.39)	17 (28.33)	10 (16.39)	8 (13.56)		
		GG	4 (6.56)	3 (5.00)	11 (18.03)	5 (8.47)		
PTPN22	rs6679677	CC	60 (98.36)	60 (100.00)	61 (100.00)	59 (100.00)	2.76	0.43
		AC	1 (1.64)	0 (0.00)	0 (0.00)	0 (0.00)		
		AA	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)		
	rs2476601	AA	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2.76	0.43
		AG	1 (1.64)	0 (0.00)	0 (0.00)	0 (0.00)		
		GG	60 (98.36)	60 (100.00)	61 (100.00)	59 (100.00)		
HLA	rs2517532	TT	19 (31.15)	7 (11.67)	14 (22.95)	8 (13.56)	11.88	0.07
		CT	27 (44.26)	32 (53.33)	25 (40.98)	35 (59.32)		
		CC	15 (24.59)	21 (35.00)	22 (36.07)	16 (27.12)		
	rs2516049	AA	45 (73.77)	39 (65.00)	46 (75.41)	47 (79.66)	6.12	0.41
		AG	14 (22.95)	15 (25.00)	12 (19.67)	11 (18.64)		
		GG	2 (3.28)	6 (10.00)	3 (4.92)	1 (1.69)		
PDE8B	rs4704397	AA	41 (67.21)	39 (65.00)	49 (80.33)	42 (71.19)	6.58	0.45
		AG	19 (31.15)	20 (33.33)	12 (19.67)	15 (25.42)		
		GG	1 (1.64)	1 (1.67)	0 (0.00)	2 (3.39)		
	rs7714529	AA	3 (4.92)	1 (1.67)	0 (0.00)	2 (3.39)	9.45	0.15
		AG	11 (18.03)	19 (31.67)	20 (32.79)	12 (20.34)		
		GG	47 (77.05)	40 (66.67)	41 (67.21)	45 (76.27)		
	rs10066802	AA	17 (27.87)	19 (31.67)	15 (24.59)	15 (25.42)	6.33	0.39
		AG	37 (60.66)	29 (48.33)	29 (47.54)	33 (55.93)		
		GG	7 (11.48)	12 (20.00)	17 (27.87)	11 (18.64)		
SH2B3	rs3184504	TT	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2.76	0.43
		CT	1 (1.64)	0 (0.00)	0 (0.00)	0 (0.00)		
		CC	60 (98.36)	60 (100.00)	61 (100.00)	59 (100.00)		
VAV3	rs4915077	TT	29 (47.54)	34 (56.67)	37 (60.66)	31 (52.54)	3.63	0.73
		CT	27 (44.26)	23 (38.33)	22 (36.07)	26 (44.07)		
		CC	5 (8.20)	3 (5.00)	2 (3.28)	2 (3.39)		
CAPZB	rs1472565	TT	23 (37.70)	16 (26.67)	28 (45.90)	26 (44.07)	11.20	0.08
		CT	28 (45.90)	39 (65.00)	24 (39.34)	23 (38.98)		
		CC	10 (16.39)	5 (8.33)	9 (14.75)	10 (16.95)		
DIO1	rs2235544	CC	21 (34.43)	19 (31.67)	13 (21.31)	17 (28.81)	3.94	0.68
		AC	30 (49.18)	29 (48.33)	33 (54.1)	37 (62.71)		
		AA	10 (16.39)	12 (20.00)	15 (24.59)	15 (25.42)		
CTLA-4	rs231775	AA	8 (13.11)	7 (11.67)	4 (6.56)	9 (15.25)	11.13	0.09
		AG	20 (32.79)	24 (40.00)	26 (42.62)	33 (55.93)		
		GG	33 (54.10)	29 (48.33)	31 (50.82)	17 (28.81)		
	rs3087243	AA	1 (1.64)	1 (1.67)	0 (0.00)	3 (5.08)	9.84	0.13
		AG	24 (39.34)	15 (25.00)	16 (26.23)	12 (20.34)		
		GG	35 (57.38)	42 (70.00)	42 (68.85)	43 (72.88)		

**Table 2.** Association between single nucleotide polymorphisms and water iodine levels (cont.)

	SNP	Genotype	Water iodine ( $\mu\text{g/L}$ , %)				$\chi^2$	<i>p</i>
			50-99	100-149	150-299	>300		
TG	rs2252696	CC	39 (63.93)	39 (65.00)	36 (59.02)	38 (64.41)	5.99	0.42
		AC	16 (26.23)	20 (33.33)	23 (37.70)	18 (30.51)		
		AA	6 (9.84)	1 (1.67)	2 (3.28)	3 (5.08)		
TSHR	rs4903957	AA	4 (6.56)	9 (15.00)	4 (6.56)	5 (8.47)	3.78	0.71
		AG	24 (39.34)	20 (33.33)	24 (39.34)	24 (40.68)		
		GG	33 (54.1)	31 (51.67)	33 (54.10)	30 (50.85)		
THRB	rs1505287	TT	15 (24.59)	23 (38.33)	13 (21.31)	12 (20.34)	8.28	0.22
		CT	31 (50.82)	28 (46.67)	31 (50.82)	29 (49.15)		
		CC	15 (24.59)	9 (15.00)	17 (27.87)	18 (30.51)		
GNAQ	rs10512065	AA	4 (6.56)	3 (5.00)	2 (3.28)	1 (1.69)	6.84	0.34
		AG	24 (39.34)	14 (23.33)	20 (32.79)	16 (27.12)		
		GG	33 (54.1)	43 (71.67)	39 (63.93)	42 (71.19)		
POU1F1	rs1976324	AA	0 (0.00)	2 (3.33)	3 (4.92)	2 (3.39)	5.37	0.50
		AG	23 (37.70)	20 (33.33)	23 (37.70)	18 (30.51)		
		GG	38 (62.3)	38 (63.33)	35 (57.38)	39 (66.10)		
PDE4D	rs27178	TT	5 (8.20)	3 (5.00)	5 (8.20)	4 (6.78)	2.78	0.84
		CT	31 (50.82)	25 (41.67)	28 (45.9)	23 (40.68)		
		CC	25 (40.98)	32 (53.33)	28 (45.9)	31 (52.54)		

**Table 4.** Knowledge of coeliac disease and gluten free food preparation amongst chefs/cooks and culinary students

SNP	Genotype	Control (%)	Case n (%)	OR (95%CI)	<i>p</i>
Thyroid antibody positive					
<i>CAPZB</i> (rs1472565)	TT	86 (41.55)	7 (20.59)	1.00	
	TC	97 (46.86)	17 (50.00)	2.15 (0.85, 5.44)	0.90
	CC	24 (11.59)	10 (29.41)	5.12 (1.76, 14.87)	<0.01*
	Dominant model			2.74 (1.14, 6.58)	0.02*
	Recessive model			3.18 (1.36, 7.45)	<0.01*
<i>PDE4D</i> (rs27178)	CC	105 (50.72)	11 (32.35)	1.00	
	CT	90 (43.48)	18 (52.94)	1.91 (0.86, 4.25)	0.91
	TT	12 (5.80)	5 (14.71)	3.98 (1.18, 13.39)	0.06
	Dominant model			2.15 (1.00, 4.64)	0.05*
	Recessive model			2.80 (0.92, 8.53)	0.07
<i>HLA</i> (rs2517532)	CC	65 (31.40)	9 (26.47)	1.00	
	CT	95 (45.89)	24 (70.59)	1.83 (0.80, 4.18)	<0.01
	TT	47 (22.71)	1 (2.94)	0.15 (0.02, 1.26)	0.04
	Dominant model			1.27 (0.56, 2.88)	0.56
	Recessive model			0.10 (0.01, 0.77)	0.03*
Subclinical hypothyroidism					
<i>PDE4D</i> (rs27178)	CC	99 (48.77)	17 (44.74)	1.00	
	CT	95 (46.80)	13 (34.21)	0.80 (0.37, 1.73)	<0.01
	TT	9 (4.43)	8 (21.05)	5.18 (1.75, 15.28)	<0.01*
	Dominant model			1.18 (0.59, 2.36)	0.65
	Recessive model			5.75 (2.06, 16.06)	<0.01*
Hypothyroxinaemia					
<i>CTLA-4</i> (rs231775)	AA	24 (10.91)	4 (13.09)	1.00	
	AG	107 (48.64)	3 (14.29)	0.17 (0.04, 0.80)	<0.01*
	GG	89 (40.45)	14 (66.67)	0.94 (0.29, 3.13)	0.09
	Dominant model			0.52 (0.16, 1.68)	0.27
	Recessive model			2.94 (1.14, 7.58)	0.03*
<i>TG</i> (rs2252696)	CC	143 (65.00)	9 (42.86)	1.00	
	CA	69 (31.36)	8 (38.10)	1.84 (0.68, 4.98)	0.41
	AA	8 (3.64)	4 (19.05)	7.94 (2.01, 31.46)	<0.01*
	Dominant model			2.48 (1.00, 6.14)	0.05*
	Recessive model			6.24 (1.70, 22.83)	<0.01*
<i>TSHR</i> (rs4903957)	GG	119 (54.09)	8 (38.10)	1.00	
	GA	84 (38.18)	8 (38.10)	1.42 (0.51, 3.93)	0.42
	AA	17 (7.73)	5 (23.81)	4.38 (1.28, 14.93)	0.02*
	Dominant model			1.92 (0.76, 4.80)	0.17
	Recessive model			3.73 (1.22, 11.43)	0.02*
Subclinical thyroid disease					
<i>PDE4D</i> (rs27178)	CC	97 (49.49)	19 (42.22)	1.00	
	CT	91 (46.43)	17 (37.78)	0.95(0.47, 1.95)	0.02
	TT	8 (4.08)	9 (20.00)	5.74(1.97, 16.77)	<0.01*
	Dominant model			1.34(0.70, 2.58)	0.38
	Recessive model			5.88(2.13, 16.24)	<0.01*

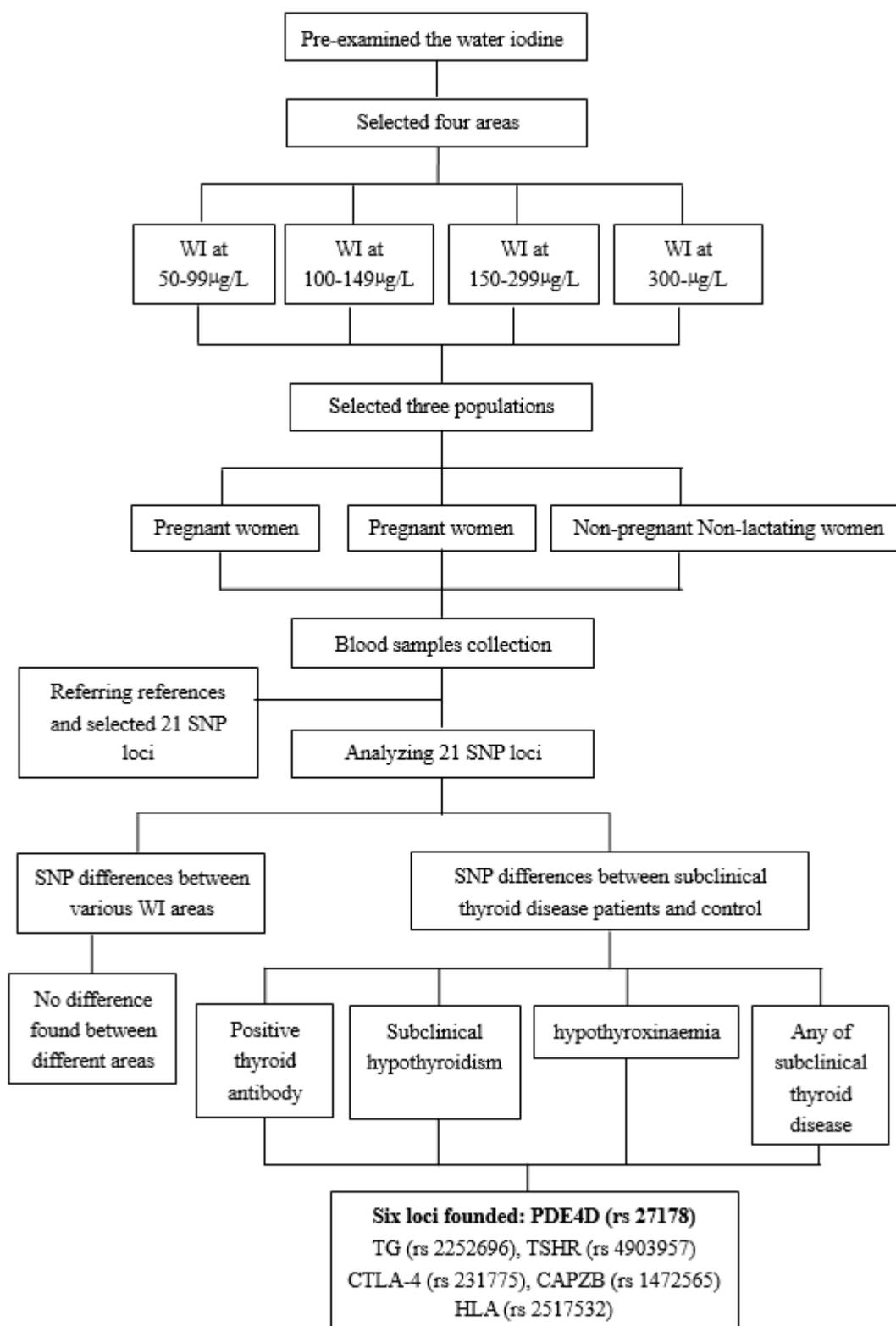


Figure 1. Flowchart of the research.

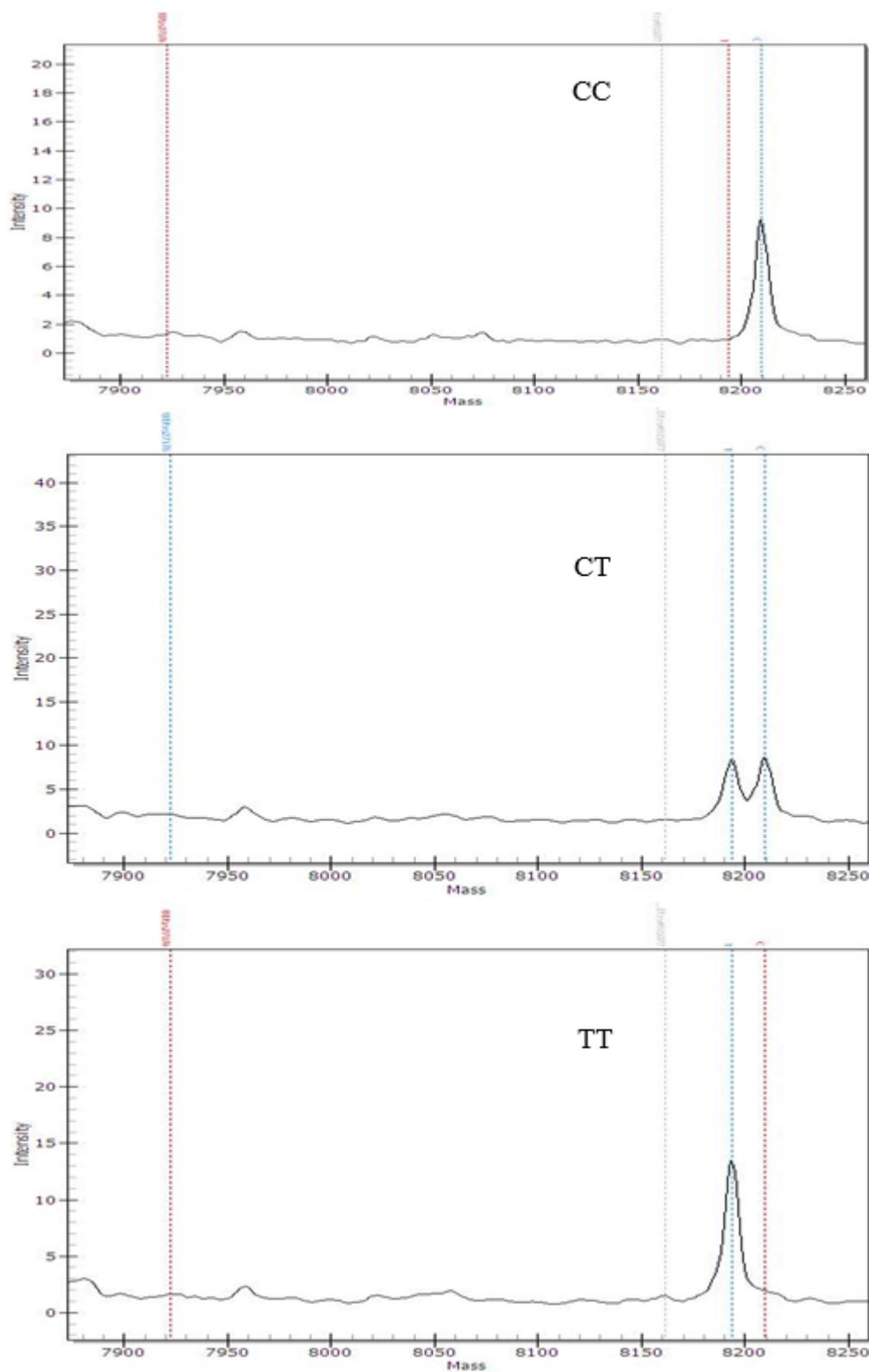


Figure 2. CC, CT, and TT genotype of PDE4D (rs27178) maps.