Longitudinal Associations Between TPO Gene Variants and TPOAb Seroconversion In A Population Based Study: Tehran Thyroid Study (TTS)

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Title page

Longitudinal Associations between TPO gene variants and TPOAb seroconversion in a population based study: Tehran Thyroid Study (TTS)

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Abstract

Background: Autoimmune thyroid diseases (AITD) are among the most common autoimmune diseases in the world. They are usually accompanied by the presence of anti-thyroid antibodies as the early predictive marker. Genetic determinants of the susceptibility to develop thyroid antibodies are still poorly understood. This study aimed to investigate the relation between thyroid peroxidase (TPO) gene variants (53 SNPs) and positive TPOAb and also to evaluate the effect of some environmental factors on changes from negative to positive TPOAb (Seroconversion).

Methods: Participants from the Tehran Thyroid Study (TTS) in phases 1 and 2 (N=5317, ≥ 20 years) were evaluated for the positive TPOAb and its relationship with 53 SNPs from TPO gene (a cross-sectional approach). At the second stage of the study (a longitudinal approach), negative TPOAb participants (control group, N= 4815) were followed up for about 5.5 (5.54±1.62) years until they have had positive results for TPOAb ("TPOAb seroconversion"). The association between TPO gene polymorphisms and TPOAb seroconversion was evaluated using logistic regression analysis and SKAT (sequence kernel association test) package.

Results: In cross-sectional analyses, 17 SNPs were associated with TPOAb positivity (521 positive TPOAb participants) after the adjustment for age, sex, body mass index (BMI), smoking, the number of parity and oral contraceptive consumption ($P < 0.05$). In longitudinal analyses, there was an association between TPOAb seroconversion and four SNPs before, and three SNPs after adjustment ($P < 0.05$).

Conclusions: TPOAb seroconversion could be affected by some thyroid peroxidase gene variants.

Keywords: Autoimmune thyroid diseases, TPOAb, seroconversion, TPO gene, single nucleotide polymorphism, SNP.

Background

Autoimmune thyroid diseases (ATIDs) are among the most prevalent type of autoimmune disorders (1). Although the exact pathogenesis of these disorders is not yet understood, there is increasing evidence in favor of a role of genetic factors in collaboration with environmental triggers (2). The basis for development of these disorders is production of antibodies against cellular and molecular structures of thyroid gland. Although thyroid peroxidase antibody (TPOAb) has not been identified as a direct cause of thyroid cell destruction, there is a strong association between TPOAb and autoimmune thyroid disorders and they are present in the serum of 90% to 95% of Hashimoto thyroiditis patients (3). This association make them a reliable serological marker for diagnosis of AITDs. The prevalence of anti-thyroid antibodies is between 5% to 24% among different communities. This prevalence has been reported above 10% in a study that has been performed in the framework of National Health and Nutrition Examination Survey (NHANES); with a prevalence of 13% for TPOAb and 11.5% for Thyroglobulin Antibody (TgAb) (4). The prevalence of TPOAb was reported 12.8% in Tehran Thyroid Study (TSS) (5).

Genetic background plays the most important role in predisposition to an autoimmune disorder (6-8). Preliminary studies for determination of genetic contribution to autoimmune thyroid disorders performed by “candidate gene identification” approach and mainly focused upon the genes having a role in the regulation of the immune system. With the introduction of the Genome-wide association studies (GWAS), it has become possible to perform genotyping on numerous individuals and at a high rate (9).

Genome-Wide Association Studies for detecting the relationship between genetic polymorphisms and levels of TPOAb in prospective cohorts are still scarce. Soo-Heon Kwak et al. in 2014 performed a two-stage GWAS on 4238 individuals along with measurement of their serum levels of TSH, T4, and TPOAb (10). They identified a novel variant of thyroid peroxidase (TPO) gene that was associated with TPOAb positivity. Two meta-analysis surveys assessed GWAS studies for demonstrating the association between genetic polymorphism and positive TPOAb and serum levels. In a meta-analysis survey by Medici et al in 2014, it was shown that the coexistence of multiple variants in an individual, considerably increases the risk of positive TPOAb and also the risk of increased levels of TSH (11). In the other meta-analysis by Matana et al in 2017, a novel polymorphism in the GRIN3A gene was significantly associated with levels of TPOAb in women (12). No study has longitudinally evaluated the association between SNPs of TPO gene and TPOAb seroconversion.

The purpose of this study was to investigate the relation between the variants in TPO locus (53 SNPs) and TPOAb positivity/seroconversion and also to evaluate the effect of some environmental factors on the conversion.

Materials and Methods

Subjects and Study design

This study was conducted in the framework of the Tehran Thyroid Study (TTS); a cohort study, being performed in the context of Tehran Lipid and Glucose Study (TLGS), to collect comprehensive information on the thyroid diseases and their long-term consequences in the population of Tehran, the capital of Iran. TLGS and TTS have been

described extensively elsewhere (13). Briefly, TTS has been started at 1997. It designed in two stages, first stage was cross-sectional (phase 1) and second was a longitudinal study (phase 2, 3, and 4). The length of each study phase was about three years and the intervals between phases were four years. A total population of TTS was 5783. In the first phase 4174 and in second phase 1609 new subjects participated. The subjects of TTS were adults (aged ≥ 20 years) with thyroid function test results (5).

In the present study, the first stage was a cross-sectional (the first phase of TTS), and the second one was a longitudinal study (phases 2-4 of TTS). The study population was TTS participants who had genotype data for selected polymorphisms of the TPO gene and also had information for TPOAb test results at baseline (first and second phase of TTS). Pregnant women (n=40) were excluded. In the cross-sectional stage, we examined the correlation of different polymorphisms genotypes of the TPO gene with positive TPOAb. In the second stage of the study, TPOAb positive subjects (n=521) were excluded from the analysis, and negative TPOAb subjects (n=4237) were examined in subsequent phases until TPOAb seroconversion (until phase 4). Flowchart of participants through the study is shown in Fig.1.

The effect of polymorphisms on seroconversion was evaluated in the presence of some probable effective factors, such as age, sex, BMI, smoking, the number of parity and the use of Oral Contraceptive pills (OCPs).

Phenotypic measurements and covariates

TPOAb measurements were performed on frozen serum samples. Measurement for all samples were done in the same day using IEMA (Immunoenzymometric assay) method

by monoband Inc. Lake Forest, CA 92630, USA kit. Inter and intra-assay CVs were 3.9% and 4.7%, respectively. The normal (negative) range defined for this kit was less than 35 IU/ml (5). Body mass index (BMI) was calculated as weight in kilograms divided by the height in meters squared. Weight and height were taken by trained health care provider and were measured according to the standard protocol. Data on Parity, smoking and OCP consumption (biphasic or triphasic contraceptive tablets) were obtained by questionnaire. Smoking status was categorized as ever (daily or sometimes consumption) and never smokers by question at questionnaire (14).

Genetic Data

Genotyping: Blood samples used for extraction of genomic DNA from peripheral lymphocytes as previously described (Truett et al., 2000). Quality and quantity of extracted DNA were assessed by electrophoresis and spectrophotometry. Genotyping was performed with Illumina Human OmniExpress-24-v1-0 bead chip containing 649,932 SNP loci (Illumina Inc., San Diego, CA, USA) (14). A total of 65 SNPs of TPO gene was recognized. After the quality check, the genotype information for selected markers was extracted from the chipped dataset for all individuals.

Quality control and genetic association

Deviation from Hardy-Weinberg equilibrium ($P<0.01$) used to filter low-quality SNPs. Forty-nine SNPs with MAF greater than 0.05 (4 SNPs had MAF<0.05) were considered for association analysis by logistic regression (Supplementary Table 1 and Supplementary figure 1). The reference alleles, for running logistic regression, were selected according to the GWAS catalog web reference (https://www.ebi.ac.uk/gwas/).

The reference homozygote genotype level was considered as the reference genotype for reporting odds ratio (OR). All models adjusted for age, sex, BMI, smoking, OCP use and number of parity as covariates, using principal components (PC1 and PC2) from the genome-wide SNP data. To calculate Hardy-Weinberg equilibrium (HWE), and statistically evaluation of genetic association, we used PLINK2 (https://www.cog-genomics.org/plink/2.0/) (genomic inflation=1.001). Sequence-based kernel machine association test (SKAT) was used to increase the study capability where the minor MAF was less than 0.05 or sparse samples. Therefore, 53 SNPs were tested by SKAT (Supplementary Table 1). SKAT model was designed and implemented in two steps. During the first step, by the Haploview software and using the Four Gamet rule, SNPs were split into linkage disequilibrium (LD) blocks. In this step, SNPs were grouped into 17 blocks (supplementary figures 2&3). In the second step, the relationship between positive TPOAb and the blocks (adjusted for age, sex, smoking, parity, BMI, and OCP consumption) and pc1 and pc2 under the SKAT model was measured.

The statistical power of two stages of the study by the SKAT package for a sample size of 5327 in the cross-sectional step (the first step) and 4531 in the second step, considering 17 LD blocks and a significant level of 0.05 was 76% and 72% for first and second stages, respectively.

Statistical Analysis

For describing the basic characteristics of the subjects, for continuous variables (age, number of parities, and body mass index), mean and standard deviation were used, for continuous variables with non-normal distribution (TPOAb level), median and interquartile range

were used and qualitative variables (gender, smoking, and OCP consumption) were reported as a percentage and numeric. To evaluate SNPs and describing allele frequency indices, MAF and heterozygosity were used. Finally, testing for deviations from HWE was also performed by the Chi-Square test. To investigate the differences leven test (for equality of variances), t-test (for equality of means), and Chi-Square test (for equality of proportions) were used. Data were analyzed using Haploview, R (SKAT and SnpStats packages) and PLINK software.

Results

Baseline characteristics of participants have been summarized and shown in the table 1. Of the 5783 participants in the Tehran Thyroid Study, 5327 subjects took part in the current study (40 pregnant women and 416 subjects without genotyping were excluded) (Figure 1). At the baseline TPOAb negative and positive subjects were 4531(85.2%) and 787(14.8%), respectively. At the first stage, with a cross-sectional approach, 49 SNPs were assessed by the logistic regression model with the outcome of positive TPOAb. Among these, 17 SNPs had a significant association with positive TPOAb, after adjustment for age, gender, smoking status, BMI, and the number of parities ($P<0.05$) (Table 2). A number of SNPs were also significant but were not included in the table due to irrational odds ratio. Age and female sex increased the probability of positive TPOAb ($OR>1$; $P<0.05$), but smoking had a protective role ($OR<1$; $P<0.05$). In longitudinal stage, among 294 subjects with seroconversion, 4 SNPs showed a significant association with TPOAb seroconversion, before adjustment for the confounder variables (rs9326161, rs13431646, rs11896517, and rs6605278) ($P<0.05$). After adjustment for age, gender, smoking, BMI, number of parities, and OCP consumption, 3 SNPs had a significant

association with TPOAb seroconversion (rs6605278, rs1126799, rs4927624) (P<0.05) (Table 3). Among these SNPs, rs6605278 showed statistically significant association before and after adjustment.

In longitudinal approach, our results showed that age and BMI had significant effect on the association between aforementioned SNPs and seroconversion. Age had a protective effect on the TPOAb seroconversion risk while BMI increased it (P<0.001). For almost all polymorphisms, the effect of the confounders was the same; Exceptions: In the two SNPs (rs9678469, rs4927616) the effect of age was not significant. In two SNPs (rs1514684, rs13431646) the effect of smoking was not significant. In one SNP (rs9678469) the effect of BMI was not significant. About number of parities, significant association (protective effect) was found only in two SNPs (rs938330 and rs13431646). And about OCP consumption, just in one SNP (rs11682968) significant association with positive TPOAb (seroconversion) was found.

Genetic analysis results

Genetic analysis using the PLINK software showed that after adjustment for the variables of age, gender, smoking, BMI, number of parities, and OCP consumption, there was no significant association between the SNPs and positive TPOAb, in either cross-sectional or longitudinal stages (Supplementary Tables 2, 3).

Using SKAT, 53 studied polymorphisms were divided into 17 blocks according to their common LD. Results of the final analysis in the cross-sectional stage showed that 2 of the blocks had a significant association with positive TPOAb (block number 4 including: rs11211644, rs1546588, rs11675342, rs11675434, rs13400534, rs11682968; P=0.009,

and block number 11 including: rs6588678, rs2048722, rs1126797, rs13430369, rs2276704, rs13431173, rs732609, rs3755551, rs9383300; P=0.015); but in longitudinal analyses, none of the blocks showed a significant association with TPOAb seroconversion (P>0.05) (Table 4). All SNPs with significant association in each stage of the study along with statistical analyses used have been summarized in table 5.

Discussion

Factors affecting the production of antibodies against thyroid structure are not yet well understood. In recent years, several studies have been performed to investigate the genetic susceptibility to raise autoimmune thyroid diseases by examination of thyroid specific and/or immune regulatory genes (6-8,15,16). In the present study we investigated the association of 53 SNP near or within TPO gene polymorphims with TPOAb positivity and also with changes from negative to positive TPOAb (TPOAb seroconversion) over the time. Our study included an adult sample of 5327 and whom all had TPOAb test results at baseline and 4 phases of TTS. We detected significant association between positive TPOAb and 21 SNPs (17 SNPs in cross-sectional and 4 SNPs in longitudinal phases) and 2 blocks of SNPs in SKAT method. Among these 21 variants, according to dbSNP (https://www.ncbi.nlm.nih.gov/snp/), 14 SNPs (rs4490233, rs13423589, rs11897977, rs1967512, rs2070882, rs6588678, rs3755551, rs938330, rs4927621, rs12465127, rs17732233, rs1126799, rs2048727, rs6605278) are reporting for the first time in association with TPOAb.

Previous GWAS studies have reported the association of many variants with TPOAb levels and/or positivity (10-12,16). Among the associated variants, a few numbers are located in or near TPO locus. Kwak et al. have identified 9 variants of TPO gene been suggestively

associated with TPOAb positivity in Koreans. There was only one variant, rs2071403 had significant association ($p=1x10^{-10}$)(10). Rs2071403 and two of the suggestively identified associated SNPs (rs11682968 and rs13400534) were included in our study; the first one deviated from HWE and failed to pass the quality control but the two last ones were in the associated block no. 11. Rs2071403 was also included in the Tomari examination (2017) and showed no significant association with TPOAb in patients with AITD, although it was associated with the development of their disease. Tomari et al. examined the relationship between 8 SNPs of TPO gene and development, severity and intractability of AITDs in Japanese patients (17). They found that the serum levels of TPOAb were significantly associated with rs2071400 and rs2048722 polymorphisms. Rs2048722 is an intronic variant and was associated with TPOAb positivity in the first stage of our study. Two SNPs; including rs732609 and rs1126797 were showed no significant association in Tomari et. al. study while both of them are located in the associated block no.4 in our study. Rs732609 has previously been reported to be associated with TPOAb levels in Iranians with subclinical hypothyroidism (18). This variant is a missense mutation in exon 12 (Thr725Pro) and could affect the interactions with heme prosthetic group in the catalytic site (19). This is conceivable that slight changes in the TPO structure occurs following single residual substitution, may trigger autoimmune reaction.

The next associated SNP from the cross stage is rs7048722 that has previously reported as associated variant (17). In our study, this intronic variant is present in the associated block no. 11.

We recognized rs11675434, which is located near the TPO gene, in block no.4. This SNP has been reported in a GWAS meta-analyses in 18,297 individuals for TPOAb-positivity and in 12,353 individuals for TPOAb serum levels (11) and showed significant association with both phenotypes ($p=1.5x10^{-6}$ & $1.4x10^{-13}$ respectively). Considering the strong association of this variant, it seems probable that this block has been recognized as associated block because of being included rs11675434. Rs2071402 and 1126799 are other associated variants in our study that have been examined in relation with TPOAb and showed no association (17). Remaining 17 associated variants of TPO gene (table 2) are reported for the first in relation with TPOAb positivity/seroconversion. Among these newly reported variants only rs13431173 is located in coding region and resulted in replacement of Methionine for Valine.

About the confounders, observations in the first stage indicate that age, female sex and higher BMI, increase the probability of positive TPOAb, but smoking has a protective role in most variants. Previously, the protective effect of smoking on developing Hashimoto's Thyroiditis and the production of anti-thyroid antibodies such as TPOAb and TgAb have been reported (20, 21). In longitudinal approach, we found that increasing age did not always increase the likelihood of TPOAb positivity. This means that the risk increases until a certain age, but then decreases. More details were noticeable in Amouzegar et al. longitudinal survey on TTS population which has showed that TPOAb seroconversion was higher in women than in men and decreased with increasing age but increased again in the elderly male population (5). This finding was different from the results of Li et al. study which showed no significant association between both age and gender with TPOAb seroconversion (22). This difference may be due to decrease in the number of subjects in

elderly population in Li study. In current study, with increasing BMI, the risk of TPOAb positivity also increases. It is suggested that weight gain increases the incidence of thyroid autoimmunity. The association between obesity and the increased prevalence of autoimmune diseases, including AITDs, has been reported in several previous studies (23-27). The observations of this study were in line with previous studies. It seems that chronic low-grade inflammation in obesity is involved in pathogenesis of autoimmune diseases such as AITDs and TPOAb positivity (26). More broadly, the lack of effect of parity on TPOAb positivity in the TTS population has been reported in a concurrent study (28). Similar results about parity and negative effect of OCP consumption has been reported in several previous studies (29-32).

In this study, we examined the association between TPO gene variants and seroconversion during about 5.5 (5.54±1.62) years follow up. This longitudinal association has not been previously examined. We detected a significant association between four variants before adjustment for covariates and 3 variants after adjustment. Rs1126799 is common between cross and longitudinally associated variants and the others are reported for the first time in regards of TPOAb.

The strengths of this study were; having a good sample size in cross-sectional approach, the prospective approach of the study for evaluation of confounder variables and acceptable length of follow-up. The longitudinal view has been conducted in very little studies and can examine the effect of the confounders on an outcome in the presence of a specific SNP. Simultaneous analysis of all SNPs of a gene together in a GWAS study is much stronger than “candidate gene” studies. The use of genetic statistical analysis methods,

especially the "Sequence Kernel Association Test", such as SKAT software used in this study, can increase the strength of statistical analysis in the field of genetics.

As limitation in this study, reducing the TPOAb positive subjects in longitudinal approach resulted in insufficient sample size for an acceptable power. Positive TPOAb levels alone would not be indicative of autoimmune thyroid disease, so it was better to use thyroid function tests to diagnose the clinical condition of the thyroid. In the present study, the extraction of genetic polymorphisms was from ChIP-PED data in GWAS test, but the number of SNPs selected for final analysis is much lower than conventional GWAS studies. Of course, conducting studies based on the whole genome would be better and have a more powerful achievement in genetic studies.

This study was performed on a gene. Further studies, based on GWAS review data, are recommended on other genes, for example, those related to thyroid structure, immune system, and non-thyroid structures, and even chromosomes. And the value will be greatly enhanced by comparing the outcomes of clinical diseases such as hypothyroidism with genetic findings. Subsequent studies based on longitudinal approach with long-term follow-up of individuals can examine more effective confounders in the expression of a gene. Undoubtedly, such studies can open a new window to Personalized (Precision) Medicine and can be used to detect, track or treat autoimmune diseases such as Hashimoto's thyroiditis.

Conclusion

For the first time, we found in a population-based study significant relationship between some TPO gene SNPs and positive TPOAb and show the effect of age, sex, and BMI as confounders on the incidence of TPOAb seroconversion.

List of abbreviations

Autoimmune thyroid diseases (ATIDs), body mass index (BMI), Genome-wide association studies (GWAS), Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), minor allele frequency (MAF), National Health and Nutrition Examination Survey (NHANES), Oral Contraceptive pills (OCPs), Tehran Lipid and Glucose Study (TLGS), Tehran Thyroid Study (TSS), Thyroglobulin Antibody (TgAb), thyroid peroxidase (TPO), thyroid peroxidase antibody (TPOAb), Sequence-based kernel association test (SKAT).

Declaration

Ethics approval and consent to participate

This study was reviewed by the Ethics Committee of the Endocrine and Metabolism Research Center of Shahid Beheshti University of Medical Sciences and its code was IR.SBMU.ENDOCRINE.REC.1398.017.

Consent for publication

All information of the participants in this study has been obtained with their knowledge and consent.

Availability of data and materials

Fundamental information about TLGS and TTS studies are available in previous published articles like reference number 14. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request after

permission of Endocrine and Metabolism Research Center of Shahid Beheshti University of Medical Science.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AG: collected database and integrated study design, result and discussion. AZ: strict supervised and made fundamental changes in article. BR: collected database, result and discussion. SJN: collected database, result and discussion. MA: analyzed data and prepared result. AA: supervised and guided study design. MSD: supervised in genetic guidance. DK and YM: guided in data analysis and result. FS and SAE: supervised. FA: strict supervised.

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Tables and figures

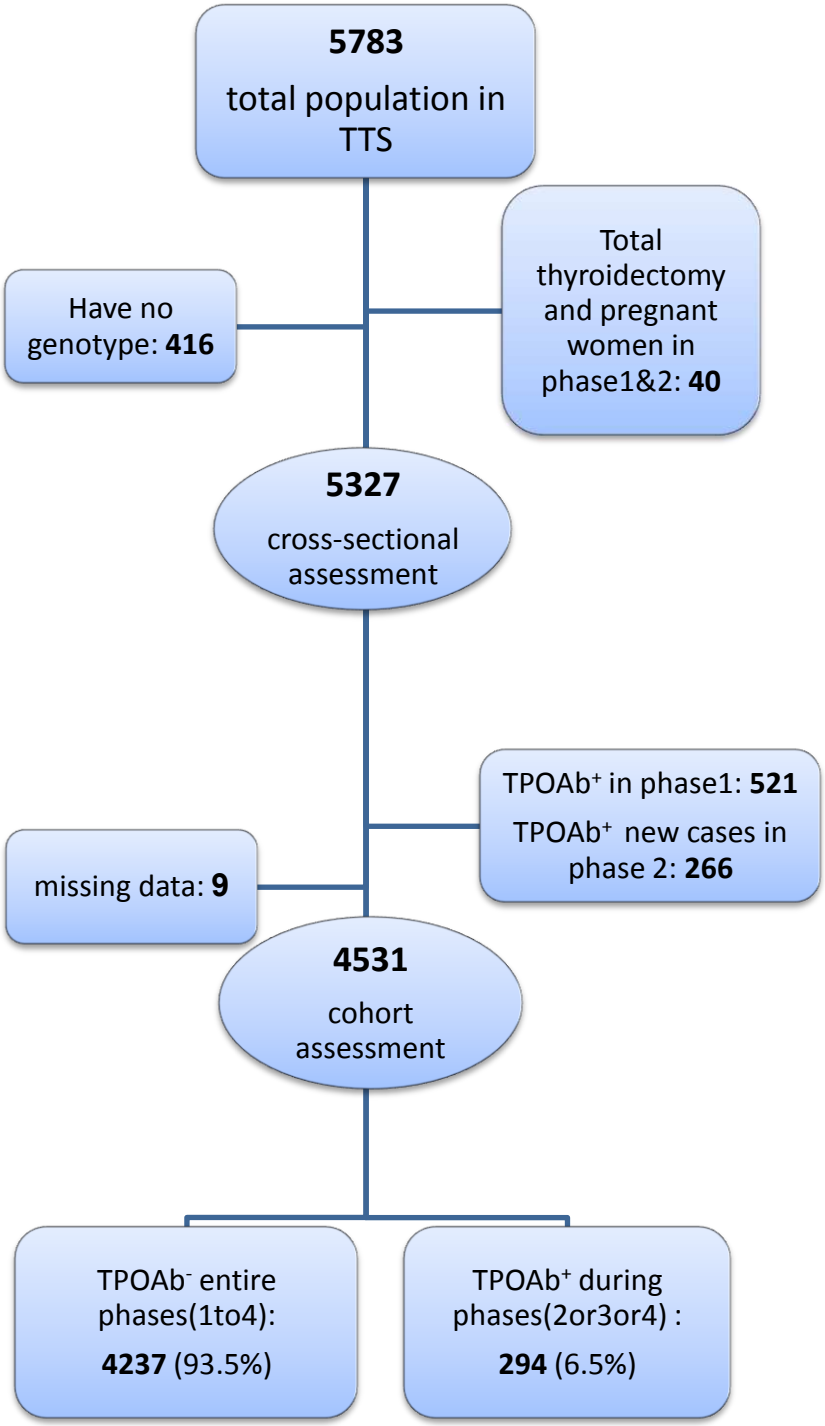

Figure 1: Flowchart of participants in Tehran Thyroid Study (TTS)

453 **Table 1: Baseline characteristics of participants based on positive and negative TPOAb beginning of phase 1**

	TPOAb negative* N= 4531(85.2%)**	TPOAb positive* N=787(14.8%)**	P-Value
Age, years, mean(± SD)	40.06(±11.66)	41.18(±13.47)	0.02
Sex, n(%)			
Male,2209(42%)	2032(91.9%)	177(8.0%)	<0.001
Female, 3109(58%)	2499(81.0%)	610(19.0%)	OR:2.62 (95%CI 2.34-3.34)
Smoking, n(٪)			
daily	290(81%)	68(19%)	0.65
no	3020(81.2%)	697(18.8%)	
sometime	1221(98.3%)	22(1.7%)	
BMI (kg/m2), mean ±SD	26.38±4.68	27.94±5.62	<0.001
Parity, number			
Mean	2.17	3.02	0.46
(min-max)	(0-13)	(0-13)	
(SD)	(2.38)	(3.04)	
OCP in women* ** n(%)			
yes	1603(79.1%)	422(20.9%)	<0.001
no	896(82.6%)	188(17.4%)	OR:1.25 (95%CI 1.04-1.51)

454 * Positive TPOAb means TPOAb≥35 IU / ml

455 ** The number and percentage of TPOAb positive and negative individuals were evaluated based on all
456 individuals in phase 1 and 2.

457 *** based on OCP use in Phase 2 (OCP consumption information in Phase 1 not available)

458

459

460 **Table 2: Significant associations between 17 SNPs and Positive TPOAb in Cross-Sectional approach after adjustement for confounders***

SNP	Genotype	frequency		OR	P-value	SNP	Variables	frequency		OR	P-value
		control	Case					control	Case		
rs4490233	(AA) ref.	403	20			**rs3755551**	(TT) ref.	334	20		
	(AG)	1869	222	2.266	**0.001**		(TC)	1729	275	2.496	**<0.001**
	(GG)	2151	538	4.879	**<0.001**		(CC)	2361	482	3.338	**<0.001**
rs13423589	(TT) ref.	117	6			**rs938330**	(CC) ref.	486	194		
	(TG)	1185	254	3.686	**0.002**		(CT)	1898	422	0.527	**<0.001**
	(GG)	3125	520	2.99	**0.01**		(TT)	2036	164	0.199	**<0.001**
rs11897977	(GG) ref.	49	4			**rs4927621**	(GG) ref.	870	80		
	(GA)	915	261	3.083	**0.034**		(GA)	2155	397	1.937	**<0.001**
	(AA)	3456	515	1.66	0.337		(AA)	1403	303	2.433	**<0.001**
rs2071402	(AA) ref.	795	22			**rs12465127**	(AA) ref	929	249		
	(AG)	2088	339	5.612	**<0.001**		(AG)	2137	374	0.632	**<0.001**
	(GG)	1534	419	10.03	**<0.001**		(GG)	1362	155	0.428	**<0.001**
rs10193983	(AA) ref.	60	107			**rs17732233**	(TT) ref.	342	229		
	(AG)	877	384	0.247	**<0.001**		(TC)	1698	393	0.317	**<0.001**
	(GG)	3488	288	0.046	**<0.001**		(CC)	2388	156	0.094	**<0.001**
rs1967512	(CC) ref.	444	175			**rs1126799**	(CC) ref.	1101	137		
	(CT)	1833	561	0.75	**0.007**		(CT)	2208	398	1.398	**0.002**
	(TT)	2148	43	0.048	**<0.001**		(TT)	1112	243	1.77	**<0.001**
rs2070882	(TT) ref.	896	278			**rs2048727**	(AA) ref.	1931	184		
	(TC)	2132	373	0.546	**<0.001**		(AG)	1946	374	0.486	**<0.001**
	(CC)	1399	129	0.29	**<0.001**		(GG)	547	205	0.247	**<0.001**
rs6588678	(GG) ref.	1899	205			**rs6605278**	(TT) ref.	116	175		
	(GA)	1965	416	1.88	**<0.001**		(TC)	1126	561	0.311	**<0.001**
	(AA)	563	157	2.512	**<0.001**		(CC)	3185	43	0.008	**<0.001**
rs2048722	(GG) ref.	966	134								
	(GA)	2162	441	1.468	**0.001**						
	(AA)	1294	204	1.176	0.19						

461 * Confounders were age, sex, BMI, smoking and number of parities. Age, gender and BMI had significant effect on positive TPOAb and smoking had
462 significant protective effect.
463

Table 3: Significant associations between 6 SNPs and TPOAb seroconversion in longitudinal study before and after adjustment for confounder variables*

SNP	Genotype	Before Adjustment				After Adjustment*			
		OR	L95	U95	P-value	OR	L95	U95	P-value
rs9326161	(TT) ref.								
	(TC)	0.287	0.076	1.081	0.065	0.292	0.051	1.686	0.169
	(CC)	0.227	0.063	0.82	**0.024**	0.202	0.037	1.092	0.063
rs13431646	(TT) ref.								
	(TC)	0.227	0.06	0.865	**0.03**	0.297	0.034	2.609	0.273
	(CC)	0.258	0.072	0.922	**0.037**	0.314	0.038	2.570	0.280
rs11896517	(CC) ref.								
	(CT)	0.444	0.191	1.032	0.059	0.651	0.166	2.555	0.538
	(TT)	0.404	0.18	0.907	**0.028**	0.461	0.122	1.744	0.254
rs6605278	(TT) ref.								
	(TC)	0.408	0.228	0.73	**0.003**	0.283	0.125	0.641	**0.002**
	(CC)	0.379	0.219	0.656	**0.001**	0.237	0.108	0.516	**<0.001**
rs4927624	(CC) ref.								
	(CT)	0.830	0.621	1.109	0.208	0.711	0.474	1.066	0.099
	(TT)	0.797	0.564	1.126	0.198	0.594	0.359	0.985	**0.044**
rs1126799	(CC) ref.								
	(CT)	0.823	0.613	1.104	0.194	0.653	0.434	0.981	**0.04**
	(TT)	0.810	0.574	1.144	0.231	0.583	0.354	0.961	**0.034**

* Confounder were age, sex, BMI, smoking, number of parities and OCP consumption.

Table 4: Association between TPO gene SNPs blocks (based on common LD) and TPOAb positivity in cross-sectional and longitudinal approaches with SKAT software

BLOCK	SNP	POSITION	MAP1*	MAP2**	BLOCK	SNP	POSITION	MAP1*	MAP2**
1	**rs4490233** **rs13423589**	1372933 1373270	0.729	0.438	**10**	**rs13431646**	1472441	0.182	0.667
2	**rs9326161** **rs4076290** **rs11897977** **rs10153889** **rs10190521** **rs1996207**	1375171 1375197 1380953 1393643 1394200 1394528	0.434	0.341	**11**	**rs6588678** **rs2048722** **rs1126797** **rs13430369** **rs2276704** **rs13431173** **rs732609** **rs3755551** **rs938330**	1479168 1492028 1494031 1494742 1495956 1496098 1496155 1498985 1502370	**0.015**	0.575
3	**rs938326**	1398009	0.094	0.554	**12**	**rs4927621** **rs12465127**	1504913 1512593	0.438	0.733
4	**rs11211644** **rs1546588** **rs11675342** **rs11675434** **rs13400534** **rs11682968**	1400723 1403306 1403856 1404043 1408111 1408458	**0.009**	0.051	**13**	**rs4927624**	1512908	0.216	0.237
5	**rs2071402** **rs10193983**	1413427 1419511	0.425	0.750	**14**	**rs13398180**	1513015	0.846	0.897
6	**rs1967512** **rs9678469**	1419728 1423335	0.061	0.351	**15**	**rs11896517**	1514340	0.259	0.629
7	**rs10519477** **rs4927606** **rs1514684**	1427818 1431524 1437406	0.878	0.995	**16**	**rs17732233** **rs1126799** **rs2048727**	1515292 1516904 1519979	0.238	0.843
8	**rs9751407** **rs7602332** **rs10204515**	1437777 1446194 1447925	0.484	0.119	**17**	**rs4927625** **rs6605278**	1521757 1543458	0.345	0.640
9	**rs4927608** **rs2070882** **rs6706775** **rs4927611** **rs4927612** **rs4927616** **rs6732480**	1449756 1454229 1455689 1456232 1456368 1459113 1459640	0.544	0.309					

MAP = Minimum Achieved P-value (significant is MAP <0.05)

*MAP in cross-sectional stage

**MAP in cohort stage

476

477 Table 5: Polymorphisms with significant association

Significant SNPs in the first stage (cross-sectional) after adjusting variables	Significant SNPs in the second stage (longitudinal) before adjusting variables	Significant SNPs in the second stage (longitudinal) after adjusting the variables	Significant SNPs in the SKAT analysis in cross-sectional stage
rs4490233	rs9326161	rs4927624	(Block 4):
rs13423589	rs13431646	rs1126799*	rs11211644
rs11897977			rs1546588
rs2071402	rs11896517	rs6605278*	rs11675342
rs10193983	rs6605278*		rs11675434
rs1967512			rs13400534
rs2070882			rs11682968
rs6588678			
rs2048722**			(Block 11):
rs3755551**			rs6588678
rs938330**			rs2048722**
rs4927621			rs1126797
rs12465127			rs13430369
rs17732233			rs2276704
rs1126799*			rs13431173
rs2048727			rs732609
rs6605278*			rs3755551**
			rs938330**

478 * Both in the cross-sectional and longitudinal study is significant.

479 ** Both in the logistic regression and in the SKAT analysis (in the cross-sectional stage) is significant.

480

481

Figures

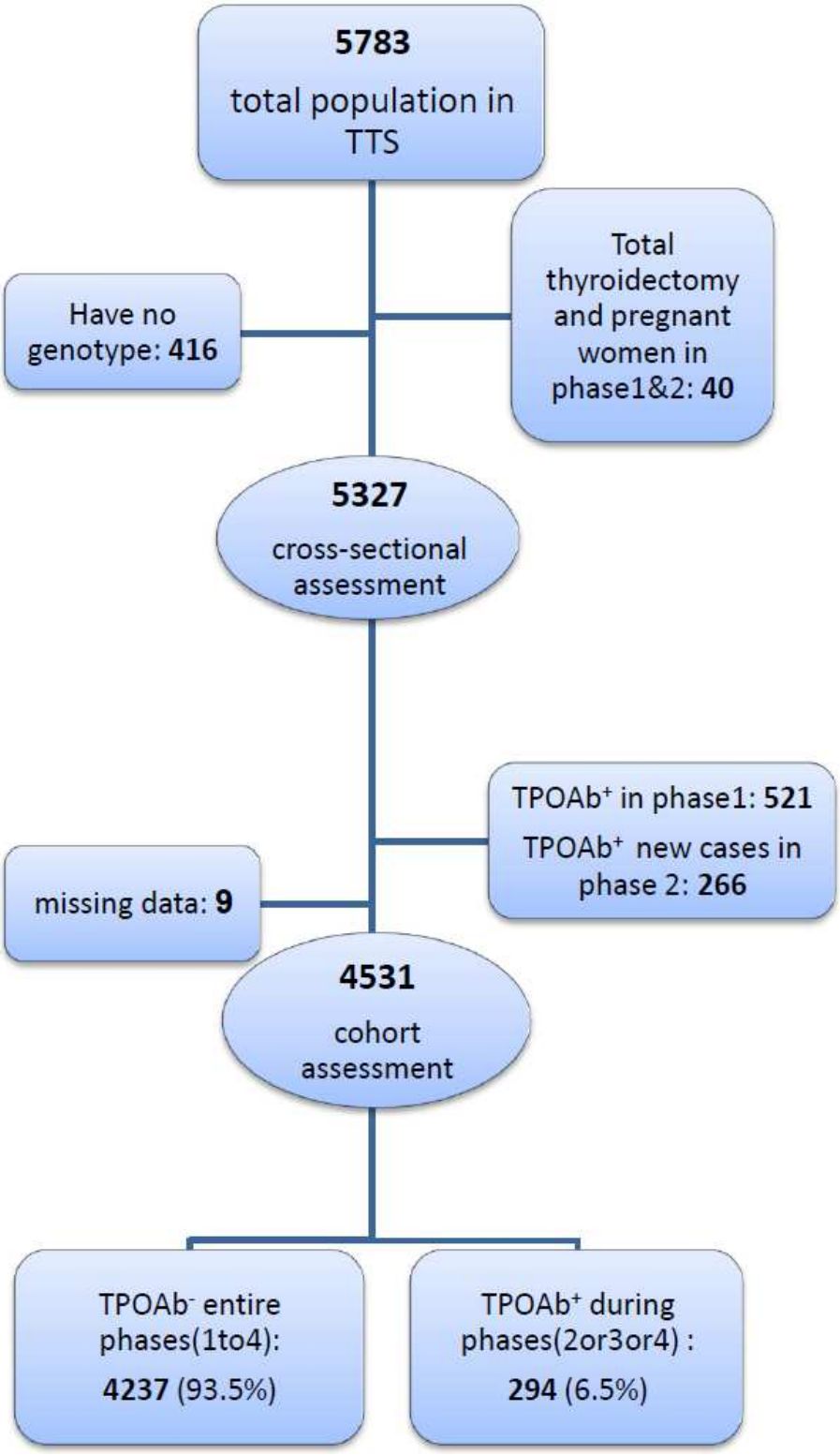

Figure 1

Flowchart of participants in Tehran Thyroid Study (TTS)

Supplementary Files

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- SupplementaryTablesandFigures.docx