Research Article

The Relationship between VEGFC Gene Polymorphisms and Autoimmune Thyroiditis

Chaoqun Gao,1,2 Jie Zhu,2 Qiu Qin,2 Xiaorong Yang,2 Yanfei Jiang,2 and Jinan Zhang3

1Graduate School of Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
2Department of Endocrinology & Rheumatology, Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, Shanghai 201318, China
3Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

Correspondence should be addressed to Jinan Zhang; zhangjinan@hotmail.com

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1. Introduction

AITDs are thyroid-specific autoimmune diseases caused by the disorder of autoimmune mechanisms, among which GD and HT are the major subtypes [1]. GD and HT have different clinical manifestations and pathophysiological characteristics. GD shows elevated TSH receptor stimulating antibody (TRAb), accompanied with lymphocyte infiltration in the thyroid gland and hypertrophy of thyroid follicular epithelial cells. HT shows interstitial fibrous tissue hyperplasia and destruction of thyroid follicles, accompanied with elevated anti-thyroglobulin antibody (TgAb) and thyroid peroxidase antibody (TPOAb). According to related research, AITDs affect about 5% of the total population and are more popular in women than in men [2]. The pathogenesis of AITDs has not been clearly studied. Genetics, immunity, and environment may be involved in the occurrence and development of AITDs [3].

Vascular endothelial growth factor (VEGF) mainly involves in neovascular diseases such as malignant tumors and plays a role in increasing vascular permeability and mediating inflammation. It is affiliated with the platelet-derived growth factor family. Angiogenesis plays a role in a variety of autoimmune inflammatory diseases, including rheumatoid arthritis [4], systemic lupus erythematosus [5], and systemic sclerosis [6]. Inhibition of angiogenesis may be a promising treatment of these diseases. VEGFs are angiogenic factors which contain five members in mammals: VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor (PGF). VEGFC is encoded in humans by the VEGFC gene, which is located on chromosome 4q34. VEGFC is a ligand of VEGF-R3 and VEGF-R2, but it exerts effects mainly through VEGF-R3 [7]. It is widely believed that VEGFC plays a major role in lymphangiogenesis and promotes the survival, growth, and migration of lymphatic endothelial cells.
2. Methods

2.1. Recruitment of the Participants. We recruited 1084 patients with AITD and 794 healthy controls from the Han Chinese population. AITD patients consisted of 256 men and 828 women. The normal healthy group was made up of 317 men and 477 women. To eliminate sampling error, all AITD patients were randomly recruited from the outpatient department of Shanghai Zhoupu Hospital, and the normal controls were consecutively enrolled from physical examination center of the same hospital. All AITD patients and normal controls were free of other autoimmune and inflammatory diseases. The participants were all from Shanghai, China. The study was permitted by the Ethics Committee of Shanghai University of Medicine & Health Sciences, and all the subjects in the study provided written informed consent.

In the AITD patients, there were 667 patients with GD (191 males and 476 females) and 417 patients with HT (65 males and 352 females). Patients with GD in the study were required to meet the following criteria: typical symptoms of hyperthyroidism, biochemical tests of hyperthyroidism, and positive TRAb [9]. Positive TPOAb or TgAb, and ultrasonographic findings of diffuse thyroid changes are the basis for the diagnosis of HT. TRAb, TgAb, TPOAb, and other serological parameters were detected by immunochemiluminescence kit (Roche Company, Switzerland) with high quality.

In order to explore the correlation between different clinical manifestations and genetic background, the relationship between SNP and various clinical subtypes was elaborated. In this study, the clinical manifestations such as Graves’ ophthalmopathy (GO) in GD patients and hypothyroidism in HT patients were included. Table 1 summarizes the clinical characteristics of all the subjects. GO, also known as thyroid-related eye disease, is characterized by exophthalmos, excessive tearing, painful eye movement, and diplopia. We can make a diagnosis based on the criteria in Williams Textbook of Endocrinology.

2.2. Isolation and Extraction of DNA Samples. Two-milliliter peripheral venous blood was collected from each individual. Patients’ DNA were extracted using Relax Gene Blood DNA System (Tiangen Biotech Co., Ltd., Beijing, China). The quality of genomic DNA was evaluated by Nano Drop 2000 spectrophotometer (Thermo Scientific Company, Waltham, USA) according to the manufacturer’s guidelines.

2.3. SNP Selection and Genotyping. This study examined the four SNPs of VEGFC (i.e., rs7664413, rs11947611, rs2046463, and rs3775194). According to previous reports, these loci are associated with several autoimmune diseases. We hypothesized that these loci are also associated with AITD susceptibility. The four SNPs need to conform to Hardy-Weinberg equilibrium (HWE) with P value greater than 0.05 and minor allele frequency (MAF) > 0.05. Genotypes of DNA samples were tested with high-throughput SNP sequencing. In simple terms, the DNA sample was amplified in 10 microliter volume. The temperature condition was set to 95°C for 15 minutes, followed by five cycles of 94°C for 30s, 60°C for 4 minutes, and 72°C for 30 seconds and 10 cycles 94°C for 30s, 60°C for 1 minute, and 72°C for 30s. The primer sequence we used is GAGTAGTCTGTTAGTTTG and GGAAACATACAAAAGGAGATGC for rs7664413. For rs11947611, the primer sequences are AACCTTGGCTCTAACTTCCT and CCTGAAACATATACAAAAAGCC; TGGTTGAGTGACTTCACTTTTGG and GGCCATGTAAAATAGAACC are the primer sequences of rs2046463. TCAACAGCTTAAGACTGAAATCAAC and TTCTGTCTAGTTCTTTGTGG and GGAAAACATAAAAGGAGATGC for rs7664413. We used SPSS (22.0, IBM, Chicago, USA) for our calculations. Measurement data are expressed as the mean plus or minus standard errors. We used SPSS (22.0, IBM, Chicago, USA) for our calculations. Measurement data are expressed as the mean plus or minus standard errors. The alleles and genotype frequency of SNP were analyzed by Chi-square test. By adjusting potential confounding factors such as thyroid function and gender, OR values before and after adjustment were calculated by multifactor logistic regression analysis to find

| Items               | AITD (%) | GD (%) | HT (%) | Controls (%) |
|---------------------|----------|--------|--------|--------------|
| Number              | 1084     | 667    | 417    | 794          |
| Age                 | 41.7 ± 14.3 | 41 ± 14.6 | 42.8 ± 13.8 | 38.9 ± 10.5 |
| Sex                 |          |        |        |              |
| Females             | 828 (76.4) | 476 (71.4) | 352 (84.4) | 477 (60.1) |
| Males               | 256 (23.6) | 191 (28.6) | 65 (15.6)  | 317 (39.9)  |
| Ophthalmopathy (+)  | /        | 99 (15.8) | /      | /            |
| Hypothyroidism (+)  | /        | /      | 174 (42.5) | /            |
|      | AITD  | NC    | P value AITD vs NC | GD  | P value GD vs NC | HT  | P value HT vs NC |
|------|-------|-------|-------------------|-----|-----------------|-----|-----------------|
| rs7664413 |       |       |                   |     |       |     |       |
| CC   | 495 (45.7) | 350 (44.1) | 0.652             | 293 (43.9) | 0.364 | 202 (48.4) | 0.238 |
| TT   | 122 (11.3)  | 85 (10.7)   | 0.779             | 87 (13.0)   | 0.357 | 35 (8.4)   | 0.281 |
| TC   | 467 (43.1)  | 359 (45.2)   | 0.572             | 288 (43.1)   | 0.296 | 180 (43.2) | 0.155 |
| C    | 1457 (67.2) | 1059 (66.7) | 0.739             | 874 (65.4) | 0.470 | 584 (70.0) | 0.095 |
| T    | 711 (32.8)  | 529 (33.3)   | 0.527             | 462 (34.6)   | 0.250 | 250 (30.0) | 0.765 |
| AA   | 410 (37.8)  | 318 (40.1)   | 0.527             | 251 (37.6)   | 0.534 | 159 (38.1) | 0.765 |
| GG   | 167 (15.4)  | 111 (14.0)   | 0.739             | 104 (15.6)   | 0.315 | 63 (15.1)  | 0.765 |
| rs11947611 |       |       |                   |     |       |     |       |
| AG   | 507 (46.8)  | 365 (46.0)   | 0.880             | 313 (46.9)   | 0.461 | 195 (46.8) | 0.461 |
| A    | 1327 (61.2) | 1001 (63.0)  | 0.255             | 815 (61.0)   | 0.259 | 513 (61.5) | 0.461 |
| G    | 841 (38.8)  | 587 (37.0)   | 0.600             | 521 (39.0)   | 0.321 | 321 (38.5) | 0.224 |
| AA   | 492 (45.4)  | 347 (43.7)   | 0.315             | 291 (43.6)   | 0.315 | 201 (48.2) | 0.470 |
| GG   | 123 (11.3)  | 85 (10.7)    | 0.600             | 88 (13.2)    | 0.35  | 35 (8.4)   | 0.765 |
| rs2046463 |       |       |                   |     |       |     |       |
| AG   | 469 (43.3)  | 362 (45.6)   | 0.880             | 289 (43.3)   | 0.841 | 181 (43.4) | 0.461 |
| A    | 1453 (67.0) | 1056 (66.5)  | 0.737             | 871 (65.2)   | 0.459 | 583 (69.9) | 0.089 |
| G    | 715 (33.0)  | 532 (33.5)   | 0.600             | 465 (34.8)   | 0.251 | 251 (30.1) | 0.596 |
| CC   | 12 (1.1)    | 4 (0.5)      | 0.029             | 8 (1.2)      | 0.092 | 4 (1.0)    | 0.052 |
| GG   | 906 (83.6)  | 636 (80.1)   | 0.399             | 553 (82.8)   | 0.354 | 354 (84.9) | 0.083 |
| rs3775194 |       |       |                   |     |       |     |       |
| CG   | 166 (15.3)  | 154 (19.4)   | 0.737             | 107 (16.0)   | 0.596 | 59 (14.1)  | 0.765 |
| C    | 190 (8.8)   | 162 (10.2)   | 0.135             | 123 (9.1)    | 0.310 | 67 (8.0)   | 0.083 |
| G    | 1978 (91.2) | 1426 (89.8)  | 1.023             | 1213 (90.9)  | 0.765 | 767 (92.0) | 0.083 |

Table 2: Associations of rs7664413, rs11947611, rs2046463, and rs3775194 in VEGFC gene with AITD, GD, and HT.

| Comparison models | Unadjusted estimates | Adjusted estimates |
|-------------------|----------------------|--------------------|
|                   | OR (95% CI)          | P value            | OR(95% CI)      | P value        |
| rs7664413         |                      |                    |                  |                |
| Allele model      | 0.98 (0.85-1.12)     | 0.74               | 0.97 (0.84-1.12) | 0.69           |
| Dominant model    | 0.94 (0.78-1.13)     | 0.50               | 0.93 (0.77-1.12) | 0.45           |
| Recessive model   | 1.06 (0.79-1.42)     | 0.71               | 1.06 (0.78-1.43) | 0.72           |
| Overdominant model| 0.92 (0.76-1.10)     | 0.36               | 0.91 (0.75-1.10) | 0.32           |
| rs11947611        |                      |                    |                  |                |
| Allele model      | 1.08 (0.95-1.23)     | 0.26               | 1.06 (0.92-1.21) | 0.43           |
| Dominant model    | 1.10 (0.91-1.32)     | 0.33               | 1.07 (0.89-1.30) | 0.47           |
| Recessive model   | 1.12 (0.86-1.45)     | 0.39               | 1.08 (0.83-1.40) | 0.59           |
| Overdominant model| 1.03 (0.86-1.24)     | 0.73               | 1.03 (0.86-1.24) | 0.74           |
| rs2046463         |                      |                    |                  |                |
| Allele model      | 0.98 (0.85-1.12)     | 0.74               | 0.97 (0.84-1.12) | 0.69           |
| Dominant model    | 0.93 (0.78-1.12)     | 0.47               | 0.93 (0.77-1.12) | 0.43           |
| Recessive model   | 1.07 (0.80-1.43)     | 0.66               | 1.06 (0.79-1.43) | 0.69           |
| Overdominant model| 0.91 (0.76-1.09)     | 0.32               | 0.90 (0.75-1.09) | 0.29           |
| rs3775194         |                      |                    |                  |                |
| Allele model      | 0.85 (0.68-1.05)     | 0.14               | 0.83 (0.66-1.05) | 0.12           |
| Dominant model    | 0.79 (0.62-1.00)     | 0.05               | 0.79 (0.62-1.00) | 0.05           |
| Recessive model   | 2.21 (0.71-6.88)     | 0.15               | 1.91 (0.60-6.08) | 0.25           |
| Overdominant model| 0.75 (0.59-0.96)     | 0.02               | 0.75 (0.59-0.96) | 0.02           |
### Table 4: Associations of four polymorphisms models in VEGFC with male AITD before and after adjusting for confounders.

| Comparison models          | Unadjusted estimates | Adjusted estimates |
|----------------------------|----------------------|--------------------|
|                            | OR (95% CI)          | P value            | OR (95% CI)          | P value            |
| rs7664413                  |                      |                    |                    |                    |
| Allele model               | 1.07 (0.83-1.37)     | 0.60               | 1.05 (0.82-1.34)    | 0.72               |
| Dominant model             | 1.01 (0.72-1.40)     | 0.96               | 0.98 (0.70-1.37)    | 0.90               |
| Recessive model            | 1.32 (0.78-2.23)     | 0.30               | 1.30 (0.77-2.21)    | 0.33               |
| Overdominant model         | 0.90 (0.65-1.26)     | 0.54               | 0.88 (0.63-1.23)    | 0.46               |
| rs11947611                 |                      |                    |                    |                    |
| Allele model               | 1.26 (0.98-1.61)     | 0.07               | 1.25 (0.97-1.60)    | 0.08               |
| Dominant model             | 1.20 (0.86-1.69)     | 0.28               | 1.16 (0.82-1.63)    | 0.40               |
| Recessive model            | 1.71 (1.04-2.82)     | 0.04               | 1.79 (1.08-2.98)    | 0.02               |
| Overdominant model         | 0.95 (0.68-1.32)     | 0.74               | 0.89 (0.62-1.24)    | 0.49               |
| rs2046463                  |                      |                    |                    |                    |
| Allele model               | 1.05 (0.82-1.35)     | 0.68               | 1.03 (0.81-1.33)    | 0.79               |
| Dominant model             | 0.98 (0.71-1.37)     | 0.92               | 0.96 (0.68-1.34)    | 0.79               |
| Recessive model            | 1.32 (0.78-2.23)     | 0.30               | 1.30 (0.77-2.21)    | 0.33               |
| Overdominant model         | 0.88 (0.63-1.23)     | 0.45               | 0.86 (0.62-1.20)    | 0.38               |
| rs3775194                  |                      |                    |                    |                    |
| Allele model               | 0.72 (0.48-1.09)     | 0.12               | 0.74 (0.49-1.12)    | 0.15               |
| Dominant model             | 0.65 (0.42-1.00)     | 0.05               | 0.66 (0.43-1.02)    | 0.06               |
| Recessive model            | ——                   | 0.03               | ——                 | 0.02               |
| Overdominant model         | 0.59 (0.38-0.92)     | 0.02               | 0.60 (0.38-0.94)    | 0.02               |

### Table 5: Allele and genotype distributions of VEGF loci in subgroups of HT patients.

|                        | Female controls | Female HT | Male controls | Male HT | Unadj/ adjust P value |
|------------------------|-----------------|-----------|---------------|--------|-----------------------|
|                        | n (%)           | n (%)     | n (%)         | n (%)  |
| rs7664413              |                 |           |               |        |
| CC                     | 207 (43.4)      | 170 (48.3)| 143 (45.1)    | 32 (49.2)| 0.831                 |
| TT                     | 54 (11.3)       | 29 (8.2)  | 31 (9.8)      | 6 (9.2) |                       |
| rs11947611             |                 |           |               |        |
| TC                     | 216 (45.2)      | 153 (43.5)| 143 (45.1)    | 27 (41.5)|                       |
| C                      | 493 (70.0)      | 630 (66.0)| 429 (67.7)    | 91 (70.0)| 0.600 0.49            |
| T                      | 211 (30.0)      | 324 (34.0)| 205 (32.3)    | 39 (30.0)|                       |
| AA                     | 185 (38.8)      | 136 (38.6)| 133 (42.0)    | 23 (35.4)| 0.120                 |
| GG                     | 80 (16.8)       | 51 (14.5) | 31 (9.7)      | 12 (18.5)|                       |
| rs2046463              |                 |           |               |        |
| AG                     | 212 (44.4)      | 165 (46.9)| 153 (48.3)    | 30 (46.1)|                       |
| A                      | 582 (61.0)      | 437 (62.1)| 419 (66.1)    | 76 (58.5)| 0.091 0.19            |
| G                      | 372 (39.0)      | 267 (37.9)| 215 (33.9)    | 54 (41.5)| 0.780                 |
| AA                     | 206 (43.2)      | 169 (48.0)| 141 (44.5)    | 32 (49.2)| 0.780                 |
| GG                     | 54 (11.3)       | 29 (8.2)  | 31 (9.8)      | 6 (9.2) |                       |
| rs3775194              |                 |           |               |        |
| AG                     | 217 (45.5)      | 154 (43.8)| 145 (45.7)    | 27 (41.5)|                       |
| A                      | 629 (65.9)      | 492 (69.9)| 427 (67.4)    | 91 (70.0)| 0.550 0.46            |
| G                      | 325 (34.1)      | 212 (30.1)| 207 (32.6)    | 39 (30.0)|                       |
| CC                     | 4 (0.8)         | 3 (0.9)   | 0 (0)         | 1 (1.5) | 0.048                 |
| GG                     | 388 (81.3)      | 297 (84.4)| 248 (78.2)    | 57 (87.7)|                       |
| rs3775194              |                 |           |               |        |
| AG                     | 85 (17.8)       | 52 (14.8) | 69 (21.8)     | 7 (10.8) |                       |
| C                      | 93 (9.7)        | 58 (8.2)  | 69 (10.9)     | 9 (6.9)  | 0.140 0.17            |
| G                      | 861 (90.3)      | 646 (91.8)| 565 (89.1)    | 121 (93.1)|                       |
out meaningful results. The correlation between VEGFC gene polymorphisms and AITD was further calculated under allele model, overdominant model, and recessive model. Linkage analysis was calculated using Haploview 4.2 software (Broad Institute, Cambridge, USA) with \( p < 0.05 \) deemed as positive.

### 3. Results

Table 2 shows the distribution of alleles and genotypes of VEGFC loci in AITD, GD, HT, and controls. No meaningful conclusions were found in the alleles and genotypes distributions of these four SNPs in GD and HT patients between cases and those in the control group. However, the genotype distribution of rs3775194 in AITD subjects (CC 1.1%, GG 83.6%, and CG 15.3%) was statistically different from that in the normal group (CC 0.5%, GG 80.1%, CG 19.4%) \( (P = 0.029) \). To further analyse the potential association between VEGFC gene polymorphisms and AITDs, we performed an analysis of four models before and after adjusting for confounding factors (age, sex). As demonstrated in Table 3, rs3775194 locus was strongly associated with AITD in overdominant model, both before and after adjusting for confounders \( (P = 0.021 \) and 0.024, respectively). No positive results were found in our study comparing GD and HT with normal controls (data are not presented).

To further investigate whether VEGFC loci are associated with AITD in different genders, as presented in Table 4, we found that rs11947611 was associated with male AITD patients in a recessive model, both before and after adjustment for confounders \( (P = 0.035 \) and 0.024, respectively). Rs3775194 was associated with male AITD patients in an overdominant model with \( P \) values of 0.017 and 0.023 before and after adjustment for confounding factors. Table 5 shows the genotypes and allele frequencies of these four loci in different sex subgroups of HT patients. The genotype distribution of rs3775194 in male HT patients (CC 1.5%, GG 87.7%, and CG 10.8%) was significantly different from that in male control group (GG 78.2% and CG 21.8%) \( (P = 0.048) \), but there were no positive results in allele frequency between the two groups. We have not yet found an association between VEGFC loci and female AITD patients (Table 6). No distinguish difference was found between the female HT and the control group in four loci.

We further analyzed the correlation between four loci of VEGFC and some clinical phenotypes. It can be seen from Table 7 that there was no correlation between four polymorphisms of VEGFC and susceptibility to Graves’ ophthalmopathy (GO). Table 8 shows that the distribution of these loci was not associated with hypothyroidism in HT patients, either.

Haploview software showed that rs2046463 and rs7664413 formed only one linkage disequilibrium (LD) region and three main haplotypes: CA, TG, and CG. However, we did not detect an association of VEGFC haplotypes CA, TG, and CG (Figure 1) with susceptibility to AITD, GD, and HT.

### 4. Discussion

Epidemiological studies have confirmed that genetic factors play an important role in AITDs, but the known genes related to AITDs cannot fully explain the role of genetic factors in AITDs. In this study, we explored the relationship between VEGFC gene polymorphisms and AITDs using allele, dominant, recessive, and overdominant models as well as different subgroups of AITD. We found that the rs3775194 locus was associated with AITD patients, but not with GD or HT patients. We further found that rs3775194 was associated with male AITD patients under the overdominant model and rs3775194 was associated with the genotype distribution of male HT patients. Rs11947611 is associated with male AITD patients under the recessive model.

VEGF is a functional glycoprotein with high biological activity. It is also called vascular permeability factor due to its strong ability to promote the differentiation and proliferation of vascular endothelial cells. VEGF gene is involved in the occurrence and development of diabetic retinopathy and cancer [10, 11]. A study of 1 919 diabetic patients with gene polymorphisms found that three SNPs (rs17697419, rs17697515, and rs2333526) of VEGFC are associated with

| rs7664413 | OR (95% CI) | \( P \) value |
|-----------|------------|---------------|
| Allele model | 0.96 (0.83-1.12) | 0.62 |
| Dominant model | 0.93 (0.76-1.13) | 0.46 |
| Recessive model | 1.02 (0.74-1.39) | 0.92 |
| Overdominant model | 0.92 (0.76-1.12) | 0.42 |

| rs11947611 | OR (95% CI) | \( P \) value |
|-----------|------------|---------------|
| Allele model | 1.08 (0.93-1.24) | 0.31 |
| Dominant model | 1.09 (0.90-1.34) | 0.38 |
| Recessive model | 1.11 (0.85-1.47) | 0.44 |
| Overdominant model | 1.03 (0.85-1.25) | 0.76 |

| rs1485766 | OR (95% CI) | \( P \) value |
|-----------|------------|---------------|
| Allele model | 1.00 (0.88-1.15) | 0.95 |
| Dominant model | 1.09 (0.88-1.36) | 0.43 |
| Recessive model | 0.92 (0.73-1.15) | 0.48 |
| Overdominant model | 1.14 (0.94-1.39) | 0.18 |

| rs2046463 | OR (95% CI) | \( P \) value |
|-----------|------------|---------------|
| Allele model | 0.97 (0.83-1.12) | 0.64 |
| Dominant model | 0.93 (0.76-1.13) | 0.46 |
| Recessive model | 1.03 (0.75-1.41) | 0.85 |
| Overdominant model | 0.92 (0.75-1.12) | 0.39 |

| rs3775194 | OR (95% CI) | \( P \) value |
|-----------|------------|---------------|
| Allele model | 0.86 (0.68-1.09) | 0.22 |
| Dominant model | 0.81 (0.63-1.04) | 0.11 |
| Recessive model | 2.17 (0.67-7.08) | 0.18 |
| Overdominant model | 0.77 (0.60-1.00) | 0.05 |
diabetic retinopathy. Rs17697515 is also specifically associated with diabetic macular edema in T2DM patients [12]. VEGFC gene also plays an important role in the development of various autoimmune diseases such as rheumatoid arthritis [4]. VEGF-C/sVEGFR-3 ratio is significantly lower in patients with Behcet’s disease than in the control group and is correlated with the course of the disease [13]. In addition, serum VEGFC values are higher in adult-onset Still’s disease, which may be a marker of disease activity [14]. On the other hand, VEGFC levels are low in patients with systemic sclerosis, and VEGFC may be a useful indicator for early prediction of pulmonary arterial hypertension in those patients [15]. Furthermore, VEGFC aggravates intestinal inflammation in mice with experimental colitis and is associated with inflammatory lymphatic formation [16]. Similarly, VEGFCs are highly expressed in salivary duct epithelial cells in patients with primary Sjogren’s syndrome, and lymphangiogenesis is active in this syndrome [17]. Based on the facts above, it is reasonable to suspect that VEGFC is involved inAITD. Therefore, this study aimed to investigate the relationship between VEGFC gene polymorphisms and AITDs.

VEGFC gene is located on chromosome 4q34.3 and has many SNPs, including rs7664413, rs11947611, rs2046463, and rs3775194. All four of them are located in the intron region. As we discussed previously, only the rs3775194 genotype distribution of the four SNPs was associated with AITD compared with the control group. Neither the allele nor genotype of four SNPs was involved in GD subgroup. Since AITDs are sex-specific diseases, we found significant differences in the genotype distribution of rs3775194 in male HT patients compared with controls. In addition, rs3775194 was significantly associated with AITD and male AITD patients under the overdominant model. Moreover, rs11947611 was associated with male AITD under the recessive model. We did not find any significant conclusions in the four SNPs of VEGFC comparing GO in GD and hypothyroidism in HT with corresponding controls. The lack of significant positive results might be due to the differences in the influence of environmental and genetic factors on the Han population and the limited number of sampled individuals. Further large population studies warrant further study.

In genomic DNA, changes in a single base may affect the amino acid sequence and ultimately affect susceptibility to diseases [18]. The phenotype is the result of environmental, genetic and other factors. Although these loci, such as rs3775194, are localized to noncoding regions, they may influence disease occurrence by regulating gene structure or expression [19]. VEGFC gene is highly expressed in

| SNPs           | Without (%) | With (%) | P value | OR (95% CI) |
|----------------|-------------|----------|---------|-------------|
| rs7664413      |             |          |         |             |
| CC            | 234 (44.4)  | 47 (47.5)| 0.212   |             |
| TT            | 64 (12.1)   | 17 (17.2)|         |             |
| TC            | 229 (43.5)  | 35 (35.4)|         |             |
| C             | 697 (66.1)  | 129 (65.2)| 0.790  | 1.044 (0.759-1.437) |
| T             | 357 (33.9)  | 69 (34.8)|         |             |
| rs11947611    |             |          |         |             |
| AA            | 192 (36.4)  | 42 (42.4)| 0.433   |             |
| GG            | 84 (15.9)   | 12 (12.1)|         |             |
| AG            | 251 (46.7)  | 45 (45.5)|         |             |
| A             | 635 (60.2)  | 129 (65.2)| 0.194  | 0.811 (0.59-1.113) |
| G             | 419 (39.8)  | 69 (34.8)|         |             |
| rs2046463     |             |          |         |             |
| AA            | 233 (44.2)  | 46 (46.5)| 0.152   |             |
| GG            | 64 (12.1)   | 18 (18.2)|         |             |
| AG            | 230 (43.6)  | 35 (35.4)|         |             |
| A             | 696 (66.0)  | 127 (64.1)| 0.607  | 1.087 (0.791-1.493) |
| G             | 358 (34.0)  | 71 (35.9)|         |             |
| rs3775194     |             |          |         |             |
| CC            | 4 (0.8)     | 3 (3.0)  | 0.153   |             |
| GG            | 440 (83.5)  | 80 (80.8)|         |             |
| CG            | 83 (15.7)   | 16 (16.2)|         |             |
| C             | 91 (8.6)    | 22 (11.1)| 0.264   | 0.756 (0.462-1.237) |
| G             | 963 (91.4)  | 176 (88.9)|        |             |
thyroid tissue [20], whereas in patients with AITD, there is an increase in lymphocyte infiltration in thyroid tissue, which suggests lymphatic hyperplasia in thyroid tissue.

Haplotype analysis is a more powerful way to prove that a gene is associated with a disease. We found a strong linkage disequilibrium in 2 SNPs between patients and controls. However, subsequent study showed no association between haplotypes and AITD, HT, or GD.

In conclusion, rs3775194 locus was associated with AITD, male AITD, and male HT patients, and rs11947611 was associated with male AITD patients. VEGFC loci is related to the immune system and may be a risk factor for AITDs.

Data Availability

The data generated during this study are available within the article and any further information can be made available upon request to the corresponding author.

Ethical Approval

Written informed consent was obtained from all participants. The present study was approved by the Ethics Committees of Shanghai University of Medicine & Health Sciences Affiliated Zhoupou Hospital (Shanghai, China).

Conflicts of Interest

The authors declare that they had no conflict of interest.
**Authors’ Contributions**

Chaoqun Gao and Jie Zhu contributed equally to this work.

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