Target region sequencing and gene polymorphism of Chinese Uyghur individuals with autoimmune thyroid diseases (AITDs)

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ABSTRACT

The autoimmune thyroid diseases (AITDs) can mainly involve complex interactions between environmental exposure and genetic susceptibility. At present, a majority of AITDs relative genes have been identified, but the results of different ethnic origin are inconsistent. Due to the special genetic characteristics of Uyghur and the unique environment of Xinjiang Uyghur Autonomous Region, the specific pathogenesis of AITDs Uyghur patients remains unknown. Our study was carried out in the group of 100 AITDs Uyghur patients (50 GD and 50 HT) and 50 Uyghur controls. DNA was extracted from peripheral blood leukocytes and region sequencing was performed to identify candidate genes and single nucleotide polymorphisms (SNPs). Following quality control, Chi-square and logistic regression tests were used for detecting the different frequencies of genotypes and alleles between cases and controls. The results of our analyses showed that the polymorphisms of TPO, TG, TSHR and PTPRC genes were associated with AITDs Uyghur patients; CD28, TPO, PTPRC and TG were related to GD; TG, STAT3 and IL2RA were connected with HT. In conclusion, our study may explore several SNPs associate with AITDs in Chinese Uyghur individuals.

INTRODUCTION

Autoimmune thyroid diseases (AITDs) are a series of typical organ-specific autoimmune endocrine disorders, among which GD (Graves' disease) and HT (Hashimoto's thyroiditis) are most common [1]. They are characterized by T-cell and B-cell infiltration of the thyroid parenchyma, which lead to production of thyrotropin receptor antibodies (TRAb) in GD and thyroid peroxidase antibodies (TPOAb) in HT. Generally, the clinical manifestation of GD is hyperthyroidism and of HT is hypothyroidism or euthyroidism [2, 3].

AITDs are multifactorial diseases in which environmental exposure and genetic susceptibility interact as principal parts toward the development [2]. Previous studies have shown that environmental triggers contribute for about 20–30% and genetic factors for about 70%-80% to the occurrence of AITDs [4, 5]. Environmental agents, such as iodine, selenium, vitamin D, radiation, cigarette smoking, viral infections, chemical contaminants and intestinal dysbiosis have been reported to be closely related to the appearance of AITDs [6–8]. Over the past decades, numerous advances have been made by using various techniques (such as candidate gene studies and whole genome screenings) to identify a large number of susceptibility genes and loci that are associated with AITDs. In 2011, Oliver J. Brand et al. had reviewed and summarized the advances in genetic study of AITDs [9]. The valued genes were classified into three groups: HLA genes, non-HLA genes (e.g., CTLA-4, SCGB3A2, PTPN22 and IL2R) and thyroid-specific genes (e.g., TSHR and TG). Matthew J. Simmonds also sorted out AITDs susceptibility loci from 1970 to 2013 [10]. Previous research have identified the associations between the polymorphisms of reported genes (CTLA4, PTPN22, FCRL3, and ZFAT) with AITDs prognosis [11]. They found the polymorphisms of CD40 and FCRL3 were associated with GD and the polymorphisms of ZFAT were associated with HT, which demonstrated that multiple genes could be common to both diseases and some were

unique. In the last few years, more and more AITDs susceptibility genes and loci have been discovered, such as AITDs with rs6479778 (located within the ARID5B gene); GD with rs12147587 (located within the NRXN3 gene) and rs2284720 (located within TSHR genes) [12]; HT with rs7537605 (located within the VAV3 gene) [13]; AITDs with polymorphisms of TRAF1 [14] and Interleukin-22 [15]. In short, identifying more important genes and loci of AITDs is urgently needed.

As we all know, Xinjiang Uyghur Autonomous Region has its own unique environment and Uyghur people in there possess their special diet and lifestyle. As the major ethnic group of Xinjiang (46.06%), the Uyghur share their special genetic characteristics [16]. To the best of our knowledge, no study has investigated the genes and loci of AITDs Uyghur patients. To better understand the etiology of AITDs, our aim of the present study is to identify novel genes and loci which are common or unique for GD and HT Uyghur patients.

RESULTS

Clinical characteristics of subjects

In our study, we collected 100 AITDs Uyghur patients (50 GD and 50 HT) and 50 Uyghur healthy controls from the People's Hospital of Xinjiang Uyghur Autonomous Region (Xinjiang, China). As shown in Table 1, the average age of GD was 40 ± 12.8, HT was 42.9 ± 10.8 and controls was 39.3 ± 12.7. In the GD group, 37 (74%) patients were females and 13 (26%) patients were males. The HT group consisted of 34 (68%) female patients and 16 (32%) male patients. In the control group, 27 (54%) individuals were females and 23 (46%) individuals were males.

Genotypic and allelic frequencies of AITDs Uyghur patients versus controls

The case-control association analysis was performed by Chi-square and logistic regression tests. Among all data, seven SNPs of four genes showed significant with AITDs at $P < 0.05$ analyzed by Chi-square (Table 2). The major mutational allele C in rs62117031, the major mutational allele T in rs62117032, the major mutational allele A in rs4278970 and the major mutational allele A in rs4927631 of TPO gene and the major mutational allele G in rs2702998 of TG gene increased in AITDs patients compared with controls. While the major mutational allele G in rs2284735 of TSHR gene and the major mutational allele C in rs16843742 of PTPRC gene decreased in AITDs patients. All of above SNPs were confirmed to HWE in controls ($P > 0.05$) (Supplementary Table 1). What's more, AITDs-controls association analysis of above SNPs were confirmed by logistic regression test (Table 3), which affirmed that the rs62117031, rs62117032, rs4278970, rs4927631, rs4278970, rs4927631, rs2702998, rs2284735 and rs16843742 were associated with AITDs patients under different inherited models.

Genotypic and allelic frequencies of GD patients versus controls

As for GD patients, nine SNPs of four genes showed significant ($P < 0.05$) (Table 4). The deletion of allele A in rs3835894, the major mutational allele A in rs1181388 and the major mutational allele T in rs3181113 of CD28 gene; the major mutational allele C in rs62117031 of TPO gene and the major mutational allele T in rs2403883 of TG gene increased in GD patients compared with controls. While the major mutational allele T in rs2013278 of CD28 gene, the major mutational allele A in rs12144388 as well as the major mutational allele A in rs1326279 of PTPRC gene and the major mutational allele T in rs4236899 of TG gene decreased in GD patients. All of above SNPs were confirmed to HWE in controls ($P > 0.05$) (Supplementary Table 2). What's more, logistic regression test showed that the rs3835894, rs1181388, rs2013278, rs3181113, rs62117031, rs12144388, rs1326279, rs4236899 and rs2403883 were related to GD patients under different inherited models (Table 5).

Genotypic and allelic frequencies of patients with HT versus controls

As for HT patients, seven SNPs of three genes showed significant ($P < 0.05$) (Table 6). The major mutational allele T in rs4736434 of TG gene; the major mutational allele T in rs10905668, the major mutational allele T in rs1107345 and the major mutational allele T in rs10905669 of IL2RA gene elevated in HT patients. While the major mutational allele C in rs957971 of STAT3 gene, the major mutational allele A in rs791587 and the major mutational allele G in rs791588 of IL2RA gene declined in HT patients. All SNPs of controls were in HWE ($P > 0.05$) (Supplementary Table 3). Logistic regression test also confirmed that the rs4736434, rs957971, rs10905668, rs791587, rs791588, rs1107345 and rs10905669 were correlated with HT patients under different inherited models (Table 7).

Genotypic and allelic frequencies of patients with HT versus GD

Additionally, we were also interested in exploring the different allelic frequencies between HT and GD. The result of Chi-square test was shown in Table 8. Five SNPs of four genes showed significant ($P < 0.05$). The major mutational allele A in rs12888772 of TSHR gene; the major mutational allele T in rs1295686 and the major mutational allele T in rs847 of IL13 gene; the major mutational allele T in rs2013278 of CD28 gene

Table 1: Clinical characteristics of AITD Uyghur patients and controls

	Controls	Graves' disease	Hashimoto's disease
n (female/male)	50 (27/23)	50 (37/13)	50 (34/16)
Age (years)	39.3 ± 12.7	40 ± 12.8	42.9 ± 10.8
(range)	(22–71)	(19–75)	(24–72)
TSH (uIU/mL)	1.93 ± 0.56	0.57 ± 2.03	8.86 ± 15.72
TgAb (IU/mL)	29.66 ± 10.00	244.92 ± 311.20	539.65 ± 625.73
TPOAb (IU/mL)	25.32 ± 5.91	250.54 ± 210.85	364.2 ± 198.5
TRAb (IU/L)	None	13.92 ± 11.67	None
Free T4 (ng/mL)	None	3.78 ± 3.44	12.85 ± 2.64
Free T3 (pg/mL)	None	11.72 ± 9.49	5.50 ± 0.86

Data are showed as mean ± standard deviation. None, not determined; TSH, thyrotropin; TgAb, anti-thyroglobulin antibody; TPOAb, thyroid peroxidase antibodies; TRAb, anti-thyrotropin receptor antibody; Free T4, free thyroxine; Free T3, free triiodothyronine.

Table 2: Allele and genotype frequencies in AITDs patients versus controls analyzed by Chi-square test

Gene	SNP	position	Allele	case	control	Chiscore	OR (95% Cl)	*P*
TPO	rs62117031	1531907	T	98 (49.5%)	68 (68%)			
			C	100 (50.5%)	32 (32%)	9.221	2.168 (1.31–3.59)	0.002392
TPO	rs62117032	1532278	C	99 (50.5%)	70 (70%)			
			T	97 (49.5%)	30 (30%)	10.27	2.286 (1.371–3.812)	0.001354
TPO	rs4278970	1522180	C	113 (56.5%)	73 (73%)			
			A	87 (43.5%)	27 (27%)	7.704	2.082 (1.234–3.51)	0.005511
TPO	rs4927631	1543390	G	105 (53.0%)	69 (70.4%)			
			A	93 (47%)	29 (29.6%)	8.171	2.107 (1.258–3.53)	0.004257
TG	rs2702998	133978711	C	120 (61.9%)	78 (78%)			
			G	74 (38.1%)	22 (22%)	7.821	2.186 (1.255–3.808)	0.005165
TSHR	rs2284735	81553786	A	116 (58.0%)	41 (41%)			
			G	84 (42.0%)	59 (59%)	7.723	0.5032 (0.3091–0.8194)	0.005451
PTPRC	rs16843742	198672299	T	167 (86.1%)	68 (72.3%)			
			C	27 (13.9%)	26 (27.7%)	7.963	0.4228 (0.2302–0.7766)	0.004774

increased in HT patients. While the major mutational allele C in rs6602368 of IL2RA gene decreased in HTs. All of above SNPs were confirmed to HWE both in HT and GD ($P > 0.05$) (Supplementary Table 4). Finally, HTs-GDs association analysis of above SNPs were confirmed by logistic regression test (Table 9). It verified that the rs12888772, rs1295686, rs847, rs2013278 and rs6602368 were associated with HT patients compared with GD patients under different inherited models.

DISCUSSION

Autoimmune thyroid diseases (AITDs) are archetypal organ-specific disorders, such as Graves' disease (GD) and Hashimoto's thyroiditis (HT), which mainly caused by genetic and environmental factors [1, 6, 17]. Although the phenotypes of GD and HT are contrasting, their pathogenesis shared immune-genetic mechanisms [18]. Indeed, the genetic function between GD and HT has not been fully investigated till now. In our current study with aim to explore special genes and loci that contribution to AITDs Uyghur individuals of Xinjiang China, target region sequencing was performed to discriminate expression profiles of particular genes and loci in comparing 100 AITDs Uyghur patients (50 GD and 50HT) with 50 healthy Uyghur individuals. Following Hardy–Weinberg, Chi-square and logistic regression tests, the data of case-control association analysis were obtained.

Table 3: Allele and genotype frequencies in AITDs patients versus controls confirmed by logistic regression test

Gene	SNP	model	Genotype	Case	Control	OR (95% CI)	*P*-value
TPO	rs62117031	Codominant	T/T	30	26	-	-
			T/C	38	16	2.058 (0.9384–4.515)	0.07165
			C/C	31	8	3.358 (1.314–8.58)	0.01137
		Dominant	T/T	30	26		
			T/C-C/C	69	24	2.492 (1.236–5.023)	0.0107
		Recessive	T/T-T/C	68	42		
			C/C	31	8	2.393 (1.006–5.697)	0.04857
		Additive	-	-	-	1.865 (1.177–2.957)	0.007998
TPO	rs62117032	Codominant	C/C	30	25	–	-
			C/T	39	20	1.625 (0.7626–3.463)	0.2084
			T/T	29	5	4.833 (1.629–14.34)	0.004515
		Dominant	C/C	30	25		
			C/T-T/T	68	25	2.267 (1.124–4.571)	0.0222
		Recessive	C/C-C/T	69	45		
			T/T	29	5	3.783 (1.363–10.5)	0.01063
		Additive	-	-	-	2.045 (1.256–3.328)	0.004003
TPO	rs4278970	Codominant	C/C	34	26	–	-
			C/A	45	21	1.639 (0.7918–3.391)	0.1832
			A/A	21	3	5.353 (1.44–19.9)	0.01227
		Dominant	C/C	34	26		
			C/A-A/A	66	24	2.103 (1.053–4.201)	0.03528
		Recessive	C/C-C/A	79	47		
			A/A	21	3	4.165 (1.178–14.72)	0.02677
		Additive	-	-	-	2.023 (1.198–3.416)	0.008381
TPO	rs4927631	Codominant	G/G	33	26	–	-
			G/A	39	17	1.807 (0.8392–3.893)	0.1305
			A/A	27	6	3.545 (1.275–9.862)	0.01532
		Dominant	G/G	33	26		
			G/A-A/A	66	23	2.261 (1.123–4.551)	0.02228
		Recessive	G/G-G/A	72	43		
			A/A	27	6	2.688 (1.027–7.032)	0.04396
		Additive	-	-	-	1.866 (1.154–3.018)	0.01096
TG	rs2702998	Codominant	C/C	41	30	–	-
			C/G	38	18	1.545 (0.7427–3.213)	0.2445
			G/G	18	2	6.585 (1.419–30.56)	0.01609
		Dominant	C/C	41	30		
			C/G-G/G	56	20	2.049 (1.023–4.103)	0.04297
		Recessive	C/C-C/G	79	48		
			G/G	18	2	5.468 (1.215–24.61)	0.02685
		Additive	-	-	-	2.029 (1.183–3.48)	0.01012
TSHR	rs2284735	Codominant	A/A	34	8	–	-
			A/G	48	25	0.4518 (0.182–1.122)	0.08677

			G/G	18	17	0.2491 (0.09018–0.6882)	0.007349
		Dominant	A/A	34	8		
			A/G-G/G	66	42	0.3697 (0.1562–0.8754)	0.02366
		Recessive	A/A-A/G	82	33		
			G/G	18	17	0.4261 (0.1961–0.9261)	0.03126
		Additive	-	-	-	0.5028 (0.3048–0.8294)	0.007093
PTPRC	rs16843742	Codominant	T/T	72	26	–	-
			T/C	23	16	0.5191 (0.238–1.132)	0.0994
			C/C	2	5	0.1444 (0.02639–0.7907)	0.0257
		Dominant	T/T	72	26		
			T/C-C/C	25	21	0.4299 (0.2065–0.895)	0.02404
		Recessive	T/T-T/C	95	42		
			C/C	2	5	0.1768 (0.03297–0.9484)	0.0432
		Additive	-	-	-	0.4488 (0.2458–0.8194)	0.009095

Table 4: Allele and genotype frequencies in GD patients versus controls analyzed by Chi-square test

Gene	SNP	position	Allele	case	control	Chiscore	OR (95% CI)	*P*
CD28	rs3835894	204576923	A	43 (44.8%)	64 (65.3%)			
			-	53 (55.2%)	34 (34.7%)	8.251	2.32 (1.301–4.138)	0.004073
CD28	rs1181388	204575951	G	49 (50%)	68 (68%)			
			A	49 (50%)	32 (32%)	6.634	2.125 (1.193–3.785)	0.01001
CD28	rs2013278	204590658	A	62 (63.3%)	36 (40%)			
			T	36 (36.7%)	54 (60%)	10.18	0.3871 (0.2149–0.6974)	0.001423
CD28	rs3181113	204601910	G	52 (53.1%)	72 (73.5%)			
			T	46 (46.9%)	26 (26.5%)	8.781	2.45 (1.346–4.458)	0.003043
TPO	rs62117031	1531907	T	45 (45.9%)	68 (68%)			
			C	53 (54.1%)	32 (32%)	9.85	2.503 (1.404–4.462)	0.001698
PTPRC	rs12144388	198658097	G	75 (76.5%)	56 (57.1%)			
			A	23 (23.5%)	42 (42.9%)	8.31	0.4089 (0.221–0.7564)	0.003944
PTPRC	rs1326279	198650513	T	75 (76.5%)	57 (57%)			
			A	23 (23.5%)	43 (43%)	8.496	0.4065 (0.2204–0.7499)	0.00356
TG	rs4236899	134116482	G	67 (68.4%)	49 (49%)			
			T	31 (31.6%)	51 (51%)	7.652	0.4445 (0.2492–0.793)	0.005672
TG	rs2403883	133999106	C	48 (51.1%)	61 (70.9%)			
			T	46 (48.9%)	25 (29.1%)	7.421	2.338 (1.262–4.332)	0.006447

It should be noted that Hardy–Weinberg test in this study was used for quality control. Generally speaking, the frequency of each inherited allele and genotype are stable, that is, in Hardy–Weinberg equilibrium (HWE) (*P* value >0.05). In our research, all SNPs of controls were confirmed to HWE ($P > 0.05$), but not all SNPs of cases were (Supplementary Tables 1 to 4), which could be precisely important to disease's causation [19]. From our data we can see that there really exists several common as well as unique arresting genes and loci as potential genetic regions for GD and HT Uyghur patients.

In our first genome screen, performed with 100 AITDs Uyghur patients (50 GD and 50 HT) and 50 Uyghur controls, seven SNPs of four genes showed evidence for linkage with AITDs Uyghur patients (Tables 2 and 3): four SNPs (rs62117031, rs62117032, rs4278970 and rs4927631) of TPO gene and one SNP (rs2702998) of TG gene elevated in AITDs patients; otherwise rs2284735 of TSHR gene and rs16843742 of PTPRC gene received an opposite consequence. These results seemed to reflect that certain loci of above genes could be associated with AITDs Uyghur patients in

Table 5: Allele and genotype frequencies in GD patients versus controls confirmed by logistic regression test

Gene	SNP	model	Genotype	Case	Control	OR (95% CI)	*P*-value
CD28	rs3835894	Codominant	A/A	11	21	-	-
			A/-	21	22	1.822 (0.7095–4.68)	0.2124
			-/-	16	6	5.091 (1.551–16.71)	0.007277
		Dominant	A/A	11	21		
			A/---/-	37	28	2.523 (1.047–6.078)	0.03915
		Recessive	A/A-A/-	32	43		
			-/-	16	6	3.583 (1.262–10.18)	0.01656
		Additive	-	-	-	2.203 (1.232–3.939)	0.007718
CD28	rs1181388	Codominant	G/G	13	23	–	-
			G/A	23	22	1.85 (0.7545–4.535)	0.1789
			A/A	13	5	4.6 (1.337–15.82)	0.01548
		Dominant	G/G	13	23		
			G/A-A/A	36	27	2.359 (1.015–5.483)	0.04613
		Recessive	G/G-G/A	36	45		
			A/A	13	5	3.25 (1.06–9.967)	0.03926
		Additive	-	-	-	2.082 (1.157–3.747)	0.01441
CD28	rs2013278	Codominant	A/A	21	8	–	-
			A/T	20	20	0.381 (0.1369–1.06)	0.06455
			T/T	8	17	0.1793 (0.05563–0.5777)	0.003989
		Dominant	A/A	21	8		
			A/T-T/T	28	37	0.2883 (0.1114–0.7461)	0.01035
		Recessive	A/A-A/T	41	28		
			T/T	8	17	0.3214 (0.1221–0.8461)	0.02154
		Additive	-	-	-	0.4225 (0.2355–0.7582)	0.00388
CD28	rs3181113	Codominant	G/G	13	25	–	-
			G/T	26	22	2.273 (0.9442–5.47)	0.06696
			T/T	10	2	9.615 (1.829–50.55)	0.007515
		Dominant	G/G	13	25		
			G/T-T/T	36	24	2.885 (1.238–6.723)	0.01413
		Recessive	G/G-G/T	39	47		
			T/T	10	2	6.026 (1.246–29.15)	0.02555
		Additive	-	-	-	2.719 (1.394–5.304)	0.003331
TPO	rs62117031	Codominant	T/T	15	26	–	-
			T/C	15	16	1.625 (0.6293–4.196)	0.3158
			C/C	19	8	4.117 (1.452–11.67)	0.007789
		Dominant	T/T	15	26		
			T/C-C/C	34	24	2.456 (1.079–5.591)	0.03235
		Recessive	T/T-T/C	30	42		
			C/C	19	8	3.325 (1.286–8.595)	0.01315
		Additive	-	-	-	1.991 (1.193–3.321)	0.008378
PTPRC	rs12144388	Codominant	G/G	28	17	–	-
			G/A	19	22	0.5244 (0.2218–1.239)	0.1413
			A/A	2	10	0.1214 (0.02371–0.6219)	0.01141
		Dominant	G/G	28	17		
			G/A-A/A	21	32	0.3984 (0.1762–0.9012)	0.02712

		Recessive	G/G-G/A	47	39		
			A/A	2	10	0.166 (0.03431–0.8028)	0.02555
		Additive	-	-	-	0.4141 (0.2194–0.7817)	0.00653
PTPRC	rs1326279	Codominant	T/T	28	18	–	-
			T/A	19	21	0.5816 (0.2467–1.371)	0.2156
			A/A	2	11	0.1169 (0.02316–0.5899)	0.009351
		Dominant	T/T	28	18		
			T/A-A/A	21	32	0.4219 (0.188–0.9469)	0.03641
		Recessive	T/T-T/A	47	39		
			A/A	2	11	0.1509 (0.03154–0.7218)	0.01788
		Additive	-	-	-	0.4243 (0.2282–0.7891)	0.006758
TG	rs4236899	Codominant	G/G	22	11	–	-
			G/T	23	27	0.4259 (0.171–1.061)	0.06685
			T/T	4	12	0.1667 (0.0435–0.6386)	0.008939
		Dominant	G/G	22	11		
			G/T-T/T	27	39	0.3462 (0.1444–0.8299)	0.01741
		Recessive	G/G-G/T	45	38		
			T/T	4	12	0.2815 (0.08384–0.9451)	0.04023
		Additive	-	-	-	0.4125 (0.2188–0.7776)	0.006189

Table 6: Allele and genotype frequencies in HT patients versus controls analyzed by Chi-square test

Gene	SNP	position	Allele	case	control	Chiscore	OR (95% Cl)	*P*
TG	rs4736434	134121121	C	59 (57.8%)	76 (76.0%)			
			T	43 (42.2%)	24 (24.0%)	7.51	2.308 (1.261–4.223)	0.006137
STAT3	rs957971	40519925	G	74 (72.5%)	48 (51.1%)			
			C	28 (27.5%)	46 (48.9%)	9.609	0.3948 (0.218–0.715)	0.001936
IL2RA	rs10905668	6092055	C	52 (51.0%)	74 (74.0%)			
			T	50 (49.0%)	26 (26.0%)	11.4	2.737 (1.514–4.946)	0.000734
IL2RA	rs791587	6088699	G	71 (71.0%)	49 (49.0%)			
			A	29 (29.0%)	51 (51.0%)	10.08	0.3924 (0.219–0.7032)	0.001496
IL2RA	rs791588	6089342	T	67 (65.7%)	45 (45.0%)			
			G	35 (34.3%)	55 (55.0%)	8.747	0.4274 (0.2423–0.754)	0.003101
IL2RA	rs1107345	6087295	G	56 (57.1%)	74 (77.1%)			
			T	42 (42.9%)	22 (22.9%)	8.723	2.523 (1.355–4.698)	0.003143
IL2RA	rs10905669	6092093	C	53 (52.0%)	72 (72.0%)			
			T	49 (48.0%)	28 (28.0%)	8.596	2.377 (1.325–4.264)	0.003368

Xinjiang China. Accumulated evidences have indicated that the polymorphisms of TPO, TG and TSHR genes could be associated with AITDs. The TPO gene is nearly 150 kb in length and regional localization to human chromosome 2p25, whose mutations have been found in patients with hypothyroidism and could be related to thyroid destruction [20, 21]. In recent decades, a number of SNPs in the TPO gene have been genotyped and different loci showed different effect on ATIDs (both GD and HT, or respectively), which further clarified the important role of TPO played in the development of AITDs [21–24]. TG is a 660-kDa glycoprotein, which accounts for about 75%-80% of the total thyroid protein [25, 26] and deserves to be a major AITDs susceptibility gene. Earlier researches have recognized that TG SNPs were related to the development of AITDs [27–31]. In 2011, Mihaela Stefan et al. revealed a new mechanism of interaction between the TG promoter SNP variant with viral infection to AITDs, which laid the foundation for further exploration [32]. However, an explicit link

Table 7: Allele and genotype frequencies in HT patients versus controls confirmed by logistic regression test

Gene	SNP	model	Genotype	Case	Control	OR (95% CI)	*P*-value
TG	rs4736434	Codominant	C/C	18	28	-	-
			C/T	23	20	1.789 (0.7704–4.154)	0.176
			T/T	10	2	7.778 (1.525–39.68)	0.01362
		Dominant	C/C	18	28		
			C/T-T/T	33	22	2.333 (1.047–5.198)	0.03815
		Recessive	C/C-C/T	41	48		
			T/T	10	2	5.854 (1.213–28.26)	0.02782
		Additive	-	-	-	2.303 (1.232–4.303)	0.008921
STAT3	rs957971	Codominant	G/G	27	12	–	-
			G/C	20	24	0.3704 (0.1502–0.9133)	0.031
			C/C	4	11	0.1616 (0.04269–0.6118)	0.007287
		Dominant	G/G	27	12		
			G/C-C/C	24	35	0.3048 (0.1295–0.7171)	0.006496
		Recessive	G/G-G/C	47	36		
			C/C	4	11	0.2785 (0.08191–0.9472)	0.04067
		Additive	-	-	-	0.3931 (0.2104–0.7345)	0.003414
IL2RA	rs10905668	Codominant	C/C	14	27	–	-
			C/T	24	20	2.314 (0.963–5.562)	0.0607
			T/T	13	3	8.357 (2.037–34.29)	0.0032
		Dominant	C/C	14	27		
			C/T-T/T	37	23	3.102 (1.354–7.109)	0.007444
		Recessive	C/C-C/T	38	47		
			T/T	13	3	5.36 (1.423–20.19)	0.01309
		Additive	-	-	-	2.692 (1.45–4.999)	0.001709
IL2RA	rs791587	Codominant	G/G	25	14	–	-
			G/A	21	21	0.56 (0.2297–1.365)	0.2022
			A/A	4	15	0.1493 (0.04142–0.5384)	0.003657
		Dominant	G/G	25	14		
			G/A-A/A	25	36	0.3889 (0.1696–0.8916)	0.02568
		Recessive	G/G-G/A	46	35		
			A/A	4	15	0.2029 (0.06189–0.6652)	0.008463
		Additive	-	-	-	0.4206 (0.2344–0.7549)	0.003705
IL2RA	rs791588	Codominant	T/T	24	12	–	-
			T/G	19	21	0.4524 (0.1785–1.147)	0.09465
			G/G	8	17	0.2353 (0.07917–0.6993)	0.009223
		Dominant	T/T	24	12		
			T/G-G/G	27	38	0.3553 (0.1518–0.8317)	0.0171
		Recessive	T/T-T/G	43	33		
			G/G	8	17	0.3611 (0.139–0.9384)	0.03659
		Additive	-	-	-	0.4822 (0.2807–0.8283)	0.008237
IL2RA	rs1107345	Codominant	G/G	17	27	–	-
			G/T	22	20	1.747 (0.7413–4.117)	0.2021

			T/T	10	1	15.88 (1.862–135.4)	0.01145
		Dominant	G/G	17	27		
			G/T-T/T	32	21	2.42 (1.067–5.491)	0.03448
		Recessive	G/G-G/T	39	47		
			T/T	10	1	12.05 (1.477–98.32)	0.02011
		Additive	-	-	-	2.584 (1.338–4.992)	0.004716
IL2RA	rs10905669	Codominant	C/C	15	25	–	-
			C/T	23	22	1.742 (0.7323–4.146)	0.2093
			T/T	13	3	7.222 (1.765–29.56)	0.00596
		Dominant	C/C	15	25		
			C/T-T/T	36	25	2.4 (1.059–5.442)	0.03607
		Recessive	C/C-C/T	38	47		
			T/T	13	3	5.36 (1.423–20.19)	0.01309
		Additive	-	-	-	2.35 (1.284–4.299)	0.00559

Table 8: Allele and genotype frequencies in HT versus GD patients analyzed by Chi-square test

Gene	SNP	position	Allele	HT	GD	Chiscore	OR (95% Cl)	*P*
TSHR	rs12888772	81578926	T	60 (58.8%)	81 (82.7%)			
			A	42 (41.2%)	17 (17.3%)	13.65	3.335 (1.733–6.42)	0.000221
IL13	rs1295686	131995843	C	47 (47.0%)	68 (69.4%)			
			T	53 (53.0%)	30 (30.6%)	10.19	2.556 (1.428–4.574)	0.001413
IL13	rs847	131996669	C	46 (47.9%)	69 (70.4%)			
			T	50 (52.1%)	29 (29.6%)	10.16	2.586 (1.433–4.667)	0.001433
CD28	rs2013278	204590658	A	41 (42.7%)	62 (63.3%)			
			T	55 (57.3%)	36 (36.7%)	8.229	2.31 (1.298–4.111)	0.004123
IL2RA	rs6602368	6062915	T	63 (61.8%)	38 (38.8%)			
			C	39 (38.2%)	60 (61.2%)	10.57	0.3921 (0.2218–0.6931)	0.001151

between TG SNPs with the complex etiology of AITDs was not yet established. So more researches are needed. Moreover, TSHR, another obvious candidate gene for AITDs, whose polymorphisms have been reported to affect the etiology of AITDs, especially of GD [33–37]. But which SNPs of TSHR can confer risk for AITDs remains an intractable problem. PTPRC (CD45) is a protein coding gene, which can encode a member of the protein tyrosine phosphatase (PTP) family. So far, even though the role of PTPRC gene plays in the AITDs is not very clear, but the genetic variations of PTPRC have been reported as risk factors for another autoimmune disease, Juvenile idiopathic arthritis (JIA) [38]. Above mentioned SNPs harboring AITDs susceptibility genes we have identified have not been reported in other researches before, suggesting AITDs Uyghur patients could be more likely to owe their special genetic characteristics.

As we mentioned earlier, GD and HT may be influenced by distinct genetic susceptibility. So in the second stage we analyzed the genotypic and allelic frequencies of Uyghur patients with GD or HT versus controls, respectively. Our data demonstrated that there actually may present distinct genes and loci compared GD Uyghur patients with HTs (Tables 4 to 7). In GD patients, nine SNPs of four genes (CD28, TPO, TG and PTPRC) showed significant and in HT patients, there were seven SNPs of three genes (TG, IL2RA and STAT3). Similarly, SNPs harboring above genes we identified have not been reported, either (Tables 4 to 7). The important claim here is that the major mutational allele C in rs62117031 of TPO gene, which was up-regulated in GD, was consistent in AITDs Uyghur patients (Tables 2 and 4). The validation of this locus will be implemented in our further study. Nevertheless, no uniform locus showed evidence for association with both GD and HT Uyghur patients, this may be due to genetic heterogeneity [39]. Consistent with previous studies, the polymorphisms of CD28 gene were considered as good candidates for GD patients [40], and the polymorphisms of STAT3 gene were good for HT [41]. Previously, the polymorphisms of IL2RA (CD25) gene was investigated in GD [42, 43] and whether it is related to the aetiological variant of HT need more identification.

Table 9: Allele and genotype frequencies in HT versus GD patients confirmed by logistic regression test

Gene	SNP	model	Genotype	HT	GD	OR (95% CI)	*P*-value
TSHR	rs12888772	Codominant	T/T	18	34	-	-
			T/A	24	13	3.487(1.44–8.443)	0.005631
			A/A	9	2	8.5(1.657–43.61)	0.01032
		Dominant	T/T	18	34		
			T/A-A/A	33	15	4.156(1.801–9.587)	0.0008392
		Recessive	T/T-T/A	42	47		
			A/A	9	2	5.036(1.029–24.64)	0.04598
		Additive	-	-	-	3.171(1.609–6.251)	0.0008589
IL13	rs1295686	Codominant	C/C	12	25	–	-
			C/T	23	18	2.662(1.056–6.708)	0.03787
			T/T	15	6	5.208(1.616–16.79)	0.005723
		Dominant	C/C	12	25		
			C/T-T/T	38	24	3.299(1.4–7.774)	0.006359
		Recessive	C/C-C/T	35	43		
			T/T	15	6	3.071(1.078–8.748)	0.03561
		Additive	-	-	-	2.337(1.314–4.156)	0.00386
IL13	rs847	Codominant	C/C	12	26	–	-
			C/T	22	17	2.804(1.104–7.12)	0.03013
			T/T	14	6	5.056(1.56–16.38)	0.006908
		Dominant	C/C	12	26		
			C/T-T/T	36	23	3.391(1.433–8.024)	0.005445
		Recessive	C/C-C/T	34	43		
			T/T	14	6	2.951(1.026–8.491)	0.04477
		Additive	-	-	-	2.329(1.306–4.155)	0.004188
CD28	rs2013278	Codominant	A/A	10	21	–	-
			A/T	21	20	2.205(0.8354–5.82)	0.1103
			T/T	17	8	4.462(1.444–13.79)	0.009376
		Dominant	A/A	10	21		
			A/T-T/T	38	28	2.85(1.162–6.992)	0.02218
		Recessive	A/A-A/T	31	41		
			T/T	17	8	2.81(1.075–7.348)	0.0351
		Additive	-	-	-	2.117(1.206–3.717)	0.009012
IL2RA	rs6602368	Codominant	T/T	18	7	–	-
			T/C	27	24	0.4375(0.1559–1.228)	0.1163
			C/C	6	18	0.1296(0.03636–0.4621)	0.001632
		Dominant	T/T	18	7		
			T/C-C/C	33	42	0.3056(0.1141–0.8182)	0.01831
		Recessive	T/T-T/C	45	31		
			C/C	6	18	0.2296(0.0819–0.6438)	0.005158
		Additive	-	-	-	0.3613(0.1916–0.6812)	0.001654

Subsequently, we performed a direct comparison of the two diseases during discovery stage to select candidate genes and loci that would distinguish between HT and GD. Our analysis suggested that the expression of TSHR SNPs rs12888772, IL13 SNPs rs1295686, IL13 SNPs rs847 and CD28 SNPs rs2013278 were significantly higher in Uyghur patients with HT than in those with GD. While IL2RA SNPs rs6602368 was lower in HT patients than GDs (Tables 8 and 9). In addition those genes noted above, previous study has also demonstrated the key role of the IL13 SNPs played in the etiology of AITDs [44]. More importantly, we identified a major mutational allele T in rs2013278 of CD28, which elevated in HT Uyghur patients, happened to decline in GDs (Tables 4 and 8). The consistency of the experimental results not only proved the accuracy of the current genetic test but pointed out the direction for our further research.

In summary, this is the first target region sequencing and genetic association analysis performed in AITDs Uyghur patients. Here we report a series of genes and loci which are shared or special between GD and HT, may provide novel insight into understanding the etiology and pathogenesis of Uyghur patients. Besides, replicated, epidemiological and functional studies are needed to validate our findings. In our further research, we are willing to collect samples as large as possible to better illustrate our findings.

Clinical participants and methods

Subjects

The case-control participants included 100 AITDs Uyghur patients (50 GD and 50 HT) and 50 Uyghur healthy controls. All of those participants were collected from the People's Hospital of Xinjiang Uyghur Autonomous Region (Xinjiang, China). All patients with GD were diagnosed by presence of clinical history of thyrotoxicosis and positive expression of thyrotropin receptor antibodies (TRAb). All patients with HT were positive for thyroid peroxidase antibodies (TPOAb) and/or antibodies against thyroglobulin (TGAb) and showed hypothyroidism or euthyroidism with palpable diffuse goiters. All healthy controls were euthyroid and had no history or any family history of thyroid disease and were negative for all kinds of thyroid autoantibodies. Written informed consent had been obtained from all participants and the clinical characteristics of AITDs patients and controls were shown in Table1.

Genomic DNA (GDNA) preparation and target region sequencing

GDNA was extracted from 2ml peripheral venous blood in an EDTA tube of each participant. The purity (optical density (OD) 260/280 was 1.8–2.0), concentration (≥50 ng/ul) and total content (>2ug) of each GDNA reached the requirements of sequencing. GDNA of each sample was checked and sheared, followed by ending repair, adenylating 3' ends and adaptor ligation according to the manufacturer's protocols. The targeted size (300–400bp) of adaptor-ligated DNA fragments were recovered using TIANGEN Gel Extraction kit (TIANGEN, Beijing, China). The PCR amplification experiment was performed to amplify the extracted DNA in 10 PCR cycles (10cycles of: 10 seconds at 98°C, 30 seconds at 60°C, 30 seconds at 72°C, 5 minutes at 72°C, Hold at 4°C). Then the GDNA library was normalized, pooled and hybridized in accordance with the manufacturer's instructions. After being purified by Agencourt AMPure XP (Beckman Coulter), enriched elution was amplified using PCR. The amplicons were checked and quantitated, and then sequenced by Illumina Hiseq2500 (Illumina, San Diego, CA, USA).

Raw data analysis

Raw data were collected and analyzed through bioinformatical resources. FastQC was performed to quality control and detect sequencing quantity. BWA (http://bio-bwa.sourceforge.net/) was used for comparing the reference genome and GATK (https://software.broadinstitute.org/gatk/best-practices/) was applied to correct the initial comparison results obtained by the BWA software. In the end, combining the phenotypic and mutated information of the samples, the genetic analysis based on family patterns or disease patterns was used for finding genes and loci associated with AITDs.

Statistical analysis

Statistical analysis was executed with SPSS version 18.0 software (SPSS, Inc., Chicago, IL, USA). For each SNP, following the assessment of Hardy–Weinberg equilibrium (HWE), Chi-square and logistic regression tests were used for detecting the different frequencies of genotypes and alleles between cases and controls. Chi-square analysis contains four hypothetical genetic models, including Codominant, Dominant, Recessive and Allele. Whereas, Logistic regression analysis contains five: Dominant, Recessive, Log-additive and HOM / HET. The association between SNPs and AITDs was firstly evaluated, and then stratified analysis were performed based on the types of AITDs. $P < 0.05$ was considered statistical criteria.

Author contributions

Xin-Ling Wang and Yan-Ying Guo conceived and designed the experiments; Xin-Ling Wang, Meng-He Wang and Yan-Ying Guo wrote the article. Su-Li Li and Jing Zhang collected and prepared the samples. Meng-He

Wang, Yuan Chen and Jamilam Mamtiming performed the experiments. Xin-Ling Wang, Yan-Ying Guo, Meng-He Wang, Su-Li Li, Jing Zhang and Yun-Zhi Luo analyzed the data.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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