Dual effect of a polymorphism in the macrophage migration inhibitory factor gene is associated with new-onset Graves disease in a Taiwanese Chinese population

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Abstract

Backgrounds: Graves disease (GD) is an autoimmune disease. Macrophage migration inhibitory factor (MIF) is a potent cytokine that plays an important role in the regulation of immune responses. Two polymorphisms in the promoter region of *MIF*, rs5844572 and rs755622, are known to affect MIF expression. The purpose of this study was to investigate the relationship between polymorphisms in the *MIF* gene promoter and the severity of GD. **Materials and Methods:** A total of 677 individuals, including 481 GD patients and 196 ethnically matched healthy controls, were genotyped to identify differences in the distribution of the *MIF* polymorphisms rs5844572 and rs755622. **Results:** Although there were no significant differences in the allele or genotype distributions among patients with different grades of goiter in GD and healthy controls, the distribution of the C allele, especially C/C genotype, of the rs755622 single nucleotide polymorphism (SNP) in *MIF*, may be as a risk factor for goiter initiation whereas a protector against development of severe goiter in patients with untreated GD ($p < 0.05$). **Conclusion:** Agoiter-developmental model incorporating genetic (*MIF* SNP rs755622) and environmental risk factors (gender, radioiodine treatment, thyroid gland surgery and vitiligo) significantly increased the prediction accuracy. Further studies are required to address the role of *MIF* polymorphisms, as well as their association with other candidate genes, in GD.

Results

Allele and genotype distribution of *MIF* in GD patients and healthy controls

The demographic information and clinical characteristics of the 481 GD patients enrolled in this study are summarized in Table 1. The frequency of the polymorphisms examined was similar to those of the Chinese and Japanese (CHB and JPT) components of HapMap. No deviation from Hardy-Weinberg equilibrium was observed for allele frequencies of the rs5844572 and rs755622 polymorphisms in the *MIF* gene ($P > 0.05$).

Table 1.Characteristics of patients with Graves disease.

Characteristic	Case number	Graves disease, goiter grade					P value
		0	1a	1b	2	3	
Female gender [n(%)]	381 (79.2)	28 (87.5)	22 (84.6)	43 (79.6)	242 (79.1)	46 (73.0)	0.513
Age [year, median (range)]	42.0 (17-87)	46.0 (27-77)	51.5 (28-71)	40.9 (23-75)	42.0 (17-87)	37.0 (17-77)	0.001
With cigarette smoking history [n(%)]	122 (23.3)	5 (15.6)	7 (26.9)	12 (22.2)	67 (21.9)	21 (33.3)	0.270
With radioiodine treatment [n(%)]	21 (4.4)	7 (21.9)	2 (7.7)	1 (1.9)	9 (2.9)	2 (3.2)	2.281×10^{-5}
With thyroid gland surgery [n(%)]	48 (10.0)	20 (62.5)	1 (3.8)	3 (5.6)	21 (6.9)	3 (4.8)	5.887×10^{-22}
With ophthalmopathy [n(%)]	202 (42.0)	14 (43.8)	5 (19.2)	22 (40.7)	131 (42.8)	30 (47.6)	0.164
With nodular hyperplasia [n(%)]	4 (12.5)	1 (3.8)	5 (9.3)	34 (11.1)	5 (7.9)	4 (12.5)	0.733
With myxedema [n(%)]	6 (1.2)	0 (0.0)	0 (0.0)	2 (3.7)	2 (0.7)	2 (3.2)	0.188
With vitiligo [n(%)]	4 (0.8)	0 (0.0)	2 (7.7)	1 (1.9)	1 (0.3)	0 (0.0)	0.002

The distribution of the *MIF* polymorphisms among patients with different severities of goiter (grades 0, 1A, 1B, 2, and 3) as well as in healthy controls were analyzed. A-priori analysis revealed that the minimum total sample size (two-tailed hypothesis) is 98 when consider the difference of GD patients with grade 0 goiter and other groups. The allele and genotype distributions of the rs5844572 and rs755622 polymorphisms in *MIF* were not significantly different among the patient and control groups ($P > 0.05$; Table 2).

Table 2. Distributions of alleles and genotypes of the *MIF* polymorphisms among patients with Graves disease and healthy controls.

Polymorphisms, n (%)	Healthy	Graves disease, goiter grade					P value
		0	1a	1b	2	3	
rs5844572 -794(CATT)$_n$							
5	142 (36.2)	23 (35.9)	15 (28.8)	39 (36.1)	200 (32.7)	41 (32.5)	0.988[a] 0.990[b]
6	192 (49.0)	32 (50.0)	27 (51.9)	54 (50.0)	315 (51.5)	63 (50.0)	
7	54 (13.8)	9 (14.1)	10 (19.2)	15 (13.9)	92 (15.0)	21 (16.7)	
8	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.8)	1 (0.8)	
5/5	19 (9.7)	2 (6.3)	2 (7.7)	9 (16.7)	29 (9.5)	5 (7.9)	0.968[a] 0.992[b]
5/6	74 (37.8)	14 (43.8)	8 (30.8)	16 (29.6)	116 (37.9)	21 (33.3)	
5/7	26 (13.3)	5 (15.6)	3 (11.5)	5 (9.3)	25 (8.2)	9 (14.3)	
5/8	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (1.6)	
6/6	48 (24.5)	7 (21.9)	7 (26.9)	15 (27.8)	74 (24.2)	17 (27.0)	
6/7	22 (11.2)	4 (12.5)	5 (19.2)	8 (14.8)	48 (15.7)	8 (12.7)	
6/8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.0)	0 (0.0)	
7/7	3 (1.5)	0 (0.0)	1 (3.8)	1 (1.9)	9 (2.9)	2 (3.2)	
7/8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	
rs755622 G>173C							
G	76 (19.4)	17 (26.6)	10 (19.2)	13 (12.0)	99 (14.7)	20 (15.9)	0.068[a] 0.100[b]
C	316 (80.6)	47 (73.4)	42 (80.8)	95 (88.0)	522 (85.3)	106 (84.1)	
G/G	128 (65.3)	19 (59.4)	17 (65.4)	43 (79.6)	222 (72.5)	44 (69.8)	0.079[a] 0.054[b]
G/C	60 (30.6)	9 (28.1)	8 (30.8)	9 (16.7)	78 (25.5)	18 (28.6)	
C/C	8 (4.1)	4 (12.5)	1 (3.8)	2 (3.7)	6 (2.0)	1 (1.6)	

[a]Comparisons among healthy individuals and the five groups of different severity of goiter.
[b]Comparisons among the five groups of different severity of goiter.

The C allele of the rs755622 SNP in *MIF* is associated with goiter severity in patients with untreated GD

We next examined the association of the polymorphisms with the severity of goiter using a stratified method of analysis, in which the patients were stratified based on whether the GD was euthyroid, untreated or treated. It is notable that both the allele and genotype distribution of the rs755622 SNP in *MIF* showed associations with the severity of goiter in patients with untreated GD (GD patients vs healthy controls, $P = 0.006$ for allele distribution and $P = 0.009$ for genotype distribution; among GD patients with goiter of different grades, $P = 0.002$ for allele distribution and $P = 0.001$ for genotype distribution; Table 3).

Table 3. Distributions of alleles and genotypes of the *MIF* polymorphisms with respect to the severity of goiter in patients with untreated Graves disease.

Polymorphisms, n (%)	Healthy	Graves disease, goiter grade					P value
		0	1a	1b	2	3	
rs5844572 -794(CATT)$_n$							
5	142 (36.2)	4 (33.3)	5 (31.3)	16 (36.4)	84 (31.3)	22 (39.3)	0.979[a] 0.974[b]
6	192 (49.0)	5 (41.7)	8 (50.0)	22 (50.0)	134 (50.0)	27 (48.2)	
7	54 (13.8)	3 (25.0)	3 (18.8)	6 (13.6)	47 (17.5)	6 (10.7)	
8	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)	1 (1.8)	
5/5	19 (9.7)	1 (16.7)	0 (0.0)	3 (13.6)	12 (9.0)	4 (14.3)	0.935[a] 0.984[b]
5/6	74 (37.8)	1 (16.7)	3 (37.5)	9 (40.9)	50 (37.3)	10 (35.7)	
5/7	26 (13.3)	1 (16.7)	2 (25.0)	1 (4.5)	9 (6.7)	3 (10.7)	
5/8	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	1 (3.6)	
6/6	48 (24.5)	1 (16.7)	2 (25.0)	4 (18.2)	29 (21.6)	8 (28.6)	
6/7	22 (11.2)	2 (33.3)	1 (12.5)	5 (22.7)	25 (18.7)	1 (3.6)	
6/8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	
7/7	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)	1 (3.6)	
7/8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	
rs755622 G>173C							
G	76 (19.4)	7 (58.3)	4 (25.0)	7 (15.9)	42 (15.7)	7 (12.5)	0.006[a] 0.002[b]
C	316 (80.6)	5 (41.7)	12 (75.0)	37 (84.1)	226 (84.3)	49 (87.5)	
G/G	128 (65.3)	1 (16.7)	4 (50.0)	16 (72.7)	95 (70.9)	21 (75.0)	0.009[a] 0.001[b]
G/C	60 (30.6)	3 (50.0)	4 (50.0)	5 (22.7)	36 (26.9)	7 (25.0)	
C/C	8 (4.1)	2 (33.3)	0 (0.0)	1 (4.5)	3 (2.2)	0 (0.0)	

Abbreviations: MIF, macrophage migration inhibitory factor.
[a]Comparisons among healthy individuals and the five groups of different severity of goiter.
[b]Comparisons among the five groups of different severity of goiter.

Table 4. Odds ratios of severity of goiter cases categorized by *MIF* polymorphism rs755622 in patients with untreated Graves disease.

Polymorphisms		Comparison to Healthy			Comparison to Grave disease, goiter grade 0			
		OR	95%CI	P value		OR	95%CI	P value
G	H vs 0	1.000		0.001				
C		5.821	1.978-18.843					
G/G		1.000		0.002				
G/C		6.400	0.652-62.812					
C/C		32.000	2.615-391.587					
G	H vs 1a	1.000		0.579	0 vs 1a	1.000		0.074
C		1.386	0.435-4.417			0.238	0.048-1.193	
G/G		1.000		0.466		1.000		0.155
G/C		2.133	0.516-8.821			0.333	0.023-4.736	
C/C		0.000	0.000-			0.000	0.000-	
G	H vs 1b	1.000		0.577	0 vs 1b	1.000		0.003
C		0.787	0.338-1.833			0.135	0.033-0.550	
G/G		1.000		0.745		1.000		0.026
G/C		0.667	0.233-1.905			0.104	0.009-1.239	
C/C		1.000	0.117-8.524			0.031	0.001-0.720	
G	H vs 2	1.000		0.221	0 vs 2	1.000		1.417×10^{-4}
C		0.773	0.511-1.169			0.133	0.040-0.438	
G/G		1.000		0.458		1.000		7.072×10^{-5}
G/C		0.808	0.495-1.321			0.126	0.013-1.254	
C/C		0.505	0.131-1.955			0.016	0.001-0.226	
G	H vs 3	1.000		0.215	0 vs 3	1.000		3.661×10^{-4}
C		0.594	0.259-1.363			0.102	0.025-0.411	
G/G		1.000		0.417		1.000		0.002
G/C		0.711	0.287-1.764			0.111	0.010-1.249	
C/C		0.000	0.000-			0.000	0.000-	

Abbreviations: MIF, macrophage migration inhibitory factor; OR, odd ratio; CI, confidence interval.

A-priori analysis revealed that the minimum total sample size (two-tailed hypothesis) is 46 when consider the difference of untreated GD patients with grade 0 goiter and other groups. Therefore the comparison between GD patients with grade 0 goiter and healthy controls, as well as GD patients with grade 0 goiter and those with grade 2 goiter, were further analyzed. Logistic regression analyses revealed that in the untreated group, GD patients with the C allele may be risk for initial goiter development (odds ratio (OR): 5.821, 95% confidence interval (CI): 1.978-18.843 for GD patients with grade 0 goiter as compared to healthy individuals), but may be protected from severe goiter development (OR: 0.133, 95%CI: 0.040-0.438 for GD patients with goiter of grade 2, as compared to those with grade 0 goiter; Table 4). In addition, the C/C genotype may be risk for initial goiter development (OR: 32.000, 95%CI: 2.615-391.587 for GD patients with grade 0 goiter as compared to healthy individuals), but protective against development of severe goiter (OR: 0.016, 95%CI: 0.001-0.226 for GD patients with goiter of grades 1b, 2 and 3, respectively, as compared to those with grade 0 goiter; Table 4). These results suggest that the C allele of the rs755622 SNP in *MIF*, especially the C/C genotype, may play a role as risk factor for goiter initiation, and may play a protective role against development of severe goiter in patients with untreated GD.

The C allele of the rs755622 SNP in *MIF* and other clinical features of Graves disease

A comparison of clinical features [gender, age, frequency of radioiodine treatment, thyroid gland surgery, ophthalmopathy, nodular hyperplasia, myxedema, vitiligo, cigarette smoking habit, as well as thyroid functions including initial FT4, TSH and anti-thyroid hormone receptor antibody (TRAb) levels amongrs755622 genotypes (G/G and G/C+C/C)] was shown. Results suggest that in the untreated group, the rs755622 SNP in *MIF* is also associated with age, thyroid gland surgery and vitiligo (Table 5). However, the rs755622 SNP in *MIF* was shown no association toward any thyroid function in the untreated group (Table 5).

Table 5. Clinical significance of *MIF* genotype in patients with untreated Graves disease.

Characteristic	rs755622 genotypes		
	G/G (n = 137)	G/C + C/C (n = 61)	P value
Female gender [n(%)]	104 (75.9)	53 (85.2)	0.138
Age [year, median (range)]	37.0 (17-77)	42.0 (22-74)	0.027
With cigarette smoking history [n (%)]	37 (71.2)	15 (28.8)	0.721
With radioiodine treatment[n (%)]	3 (2.2)	2 (3.3)	0.652
With thyroid gland surgery [n (%)]	3 (2.2)	5 (8.2)	0.047
With ophthalmopathy[n(%)]	59 (43.1)	24 (39.3)	0.624
With nodular hyperplasia [n(%)]	8 (5.8)	6 (9.8)	0.311
With myxedema [n(%)]	0 (0.0)	1 (1.6)	0.133
With vitiligo[n(%)]	0 (0.0)	2 (3.3)	0.033
FT4 (ng/dL)[mean (SD)]	2.2 (1.5)	2.1 (1.5)	0.717
TSH (mIU/L)[mean (SD)]	2.9 (13.8)	2.5 (14.0)	0.857
TRAb (%)[mean (SD)]	52.0 (24.3)	49.0 (23.6)	0.588

Abbreviations: MIF, macrophage migration inhibitory factor; FT4, free thyroxine; TSH,thyroid stimulating hormone; TRAb, anti-thyroid hormone receptor antibody; SD, standard deviation.

Multifactor dimensionality reduction analysis

To extend the previous findings, the interaction between rs755622 SNP in *MIF* and other non-genetic factors on the severity of goiter (0 vs 1a/1b/2/3) in untreated GD patients was determined by using the MDR analysis. The results suggested that as compared to the one-factor model, rs755622 genotype of *MIF* (G/G, G/C + C/C) (testing balance accuracy: 77.08%, OR (95%CI):12.143 (1.387-106.298), $P = 0.0047$), the 5-factor model consisted of the aforementioned genotype and the additional factors including gender, radioiodine treatment, thyroid gland surgery, and vitiligo showed to increase the prediction accuracy (testing balance accuracy: 86.72%, OR (95%CI): ∞, $P < 1.000 \times 10^{-4}$). The interaction dendrogram was shown in Figure 1.

Figure 1.Interaction dedrogram. The location of the longitudinal connecting bars indicates the strength of the dependence: left is weaker and right is stronger. The hierarchical cluster analysis placed*MIF* SNP rs755622 genotype (GG vs GC+CC) and radioiodine treatment on the same branch, demonstrating the most strong interaction between these two factors. Gender, thyroid gland surgery and vitiligo are also included in the dendrogram as shown. *MIF*, macrophage migration inhibitory factor; SNP, single nucleotide polymorphism.

Summary

In summary, our findings provide new information pertaining to the role of *MIF* gene polymorphisms and show that the rs755622 SNP is associated with the severity of goiter in patients with untreated GD in a Taiwanese Chinese population. These data suggest the need for additional studies in larger cohorts to address the role of *MIF* polymorphisms in the pathogenesis of GD as well as their relationship with other candidate genes with known/putative functions in GD.