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IMPLICATION OF *VDR* rs7975232 AND *FCGR2A* rs1801274 GENE POLYMORPHISMS IN THE RISK AND THE PROGNOSIS OF AUTOIMMUNE THYROID DISEASES IN THE TUNISIAN POPULATION

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ABSTRACT

Hashimoto's thyroiditis (HT) and Graves' disease (GD) are autoimmune thyroid diseases (AITD) that cause hypothyroidism and hyperthyroidism, respectively. The vitamin D receptor (VDR) and the Fcγ receptor IIA (FcγRIIA), are implicated in the etiology of AITD. This study was conducted to examine the implication of *VDR* rs7975232 and *FCGR2A* rs1801274 variations in the susceptibility and the prognosis of AITD in the Tunisian population. The rs7975232 and rs1801274 (R131H) polymorphisms were analyzed in 162 controls and 162 AITD patients (106 HT and 56 GD) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification of refractory mutation system-PCR (ARMS-PCR), respectively. No significant difference was demonstrated for the rs7975232 between patients and controls. However, a significant association was shown between the rs1801274 polymorphism and AITD or HT in the dominant (p = 0.03 or p = 0.01), codominant (p = 0.019 or p = 0.026) and allelic (p = 0.011 or p = 0.012) models. The rs7975232 was associated with the absence or the presence of anti-thyroglobulin antibody, with the age of AITD and GD patients during the first diagnosis (p = 0.01 and p = 0.009, respectively) and with a high T4 level at the beginning of HT disease. However, the *FCGR2A* gene polymorphism was associated with a low T4 level at the beginning of GD disease. In conclusion, this study indicates that only the *FCGR2A* variation could be related to AITD and HT susceptibility and that *VDR* and *FCGR2A* gene variations constitute factors to prognosticate the severity of AITD, HT and GD.

Keywords: Anti-thyroglobulin (anti-Tg) antibodies; Autoimmune thyroid diseases (AITD); Fcγ receptor IIA (FcγRIIA); Tetraiodothyroxine; Vitamin D receptor (VDR).

INTRODUCTION

Hashimoto's thyroiditis (HT) and Graves' disease (GD) are autoimmune thyroid diseases (AITD) that are due to a loss of self-tolerance to thyroid antigens, such as thyroglobulin (Tg), thyroid peroxidase (TPO) and thyroid-stimulating hormone receptor (TSHR), succeeded by infiltration of the thyroid by lymphocytes [1,2]. In HT, T cells cause apoptosis in follicular cells leading to thyroid destruction and hypothyroidism [3]. In GD, B cells produce immunoglobulin G (IgG) autoantibodies against TSHR, resulting in uncontrolled hormone production and hyperthyroidism [4]. Recently, Giovinazzo *et al.* [5] revealed that AITD patients have low vitamin D level compared to control individuals. The vitamin D active form binds to the nuclear vitamin D receptor (VDR) and regulates genes transcription in a cell-specific fashion and inhibits inflammatory cytokine production [6-8]. Diverse investigations have demonstrated a relationship between autoimmune thyroid susceptibility and *VDR* gene polymorphisms in various ethnic groups [9,10]. Of these polymorphisms, the rs7975232 [restriction fragment length polymorphism (RFLP) *Apa*I] in the 3' untranslated region (3'UTR) of the *VDR* gene, may modify the expression of this gene by modulating the stability of the mRNA [11].

The Fcγ receptor RIIA (FcγRIIA) protein encoded by the *FCGR2A* gene is a receptor for the fragment crystalizable (Fc) region of IgG. The FcγRIIA has a role in immunity as it links the humoral and cellular responses

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and inhibits the B cell activation [12,13]. It is expressed on the surface of thyrocytes in patients with GD [14]. Several polymorphic sites were determined on the *FCGR2A* gene. The rs1801274 polymorphism (R131H) is a substitution of an adenine (A) by a guanine (G), in the fourth exon of the *FCGR2A* gene, which causes a variation of a single amino acid histidine or arginine at the 131 position (H131 or R131) of the FcγRIIA. This polymorphism is functional as the receptor corresponding to the H131 allele had a better affinity for IgG2 than the receptor corresponding to the R131 allele [15]. The purpose of the present case-control study was to evaluate if *VDR* rs7975232 and *FCGR2A* rs1801274 gene polymorphisms would be genetic markers to predict the risk and the prognosis of AITD.

MATERIALS AND METHODS

Study Subjects. The present investigation was performed on 162 controls, 106 patients with HT and 56 patients with GD, collected from the central region of Tunisia. The clinical information of the patients was retrieved from their medical records at the Department of Endocrinology, Fattouma Bourguiba University Hospital in Monastir, Tunisia. The diagnosis of the GD and HT were determined by the usual criteria. Decreased serum thyroid stimulating hormone (TSH) (<0.15 mIU/L) and elevated serum free thyroxine (FT4) (>25.0 pmol/L) allowed diagnosis of GD. Increased serum TSH (>5.0 mIU/L), decreased serum FT4 (<8.6 pmol/L) and positive serum antibodies to thyroid peroxidase (TPO), and thyroglobulin (Tg) allow diagnosis of HT. Informed consent was obtained from all the patients and the control population. The study protocol was accepted by the Ethics Committee of Fattouma Bourguiba University Hospital, Monastir, Tunisia.

Genetic Analyses of *VDR* rs7975232 and *FCGR2A* rs1801274 Polymorphisms. DNA extraction from whole blood was performed by the salting-out method [16]. The rs7975232 polymorphism was genotyped by the polymerase chain reaction-RFLP (PCR-RFLP) technique. Specific primers, forward 5'-CAG AGC ATG GAC AGG GAG CAA-3' and reverse 5'-AGG CGG TCC TGG ATG GCC TC-3', were determined by the Primer3 program (http:// bioinfo.ut.ee/primer3/). Enzymatic digestion of the PCR product was performed with the *Apa*I restriction enzyme and the digested product was revealed on 3.0% agarose gel (Figure 1).

The *FCGR2A* gene rs1801274 polymorphism was genotyped by amplification of refractory mutation system-PCR (ARMS-PCR) technique. Specific primers, forward 5'-AAA TCC CAG AAA TTC TCA CG-3' for the R131 allele, forward 5'-AAA TCC CAG AAA TTC TCA CA-3' for the H131 allele and a common reverse primer 5'-CAC TCC TCT TTG CTC CAG TG-3' were used as previously

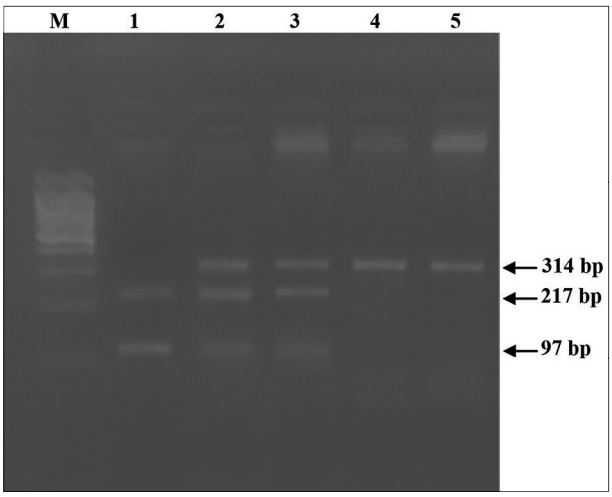

Figure 1. Vitamin D receptor rs7975232 polymorphism analysis. Lane M: molecular weight marker (100 bp DNA Ladder); Lane 1: homozygote (C/C); lanes 2 and 3: heterozygotes (C/A); lanes 4 and 5: homozygotes (A/A).

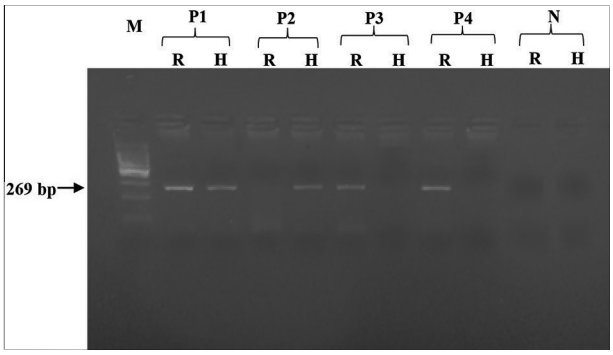

Figure 2. The *FCGR2A* rs1801274 polymorphism analysis. Lane M: molecular weight marker (100 bp DNA ladder); lane N: negative control; lanes P1: patient 1, heterozygote (R/H); lanes P2: patient 2, homozygote (H/H); lanes P3 and P4: patients 3 and 4, homozygotes (R/R).

described [17]. The resulting PCR product of 269 bp was run on 2.0% agarose gel (Figure 2).

Thyroid Function and Autoantibodies. Patients sera were collected at the beginning of the disease. Tetra-iodo-thyroxine (T4), TSH, anti-thyroglobulin (anti-Tg) and anti-TPO levels were determined by the Clinical Chemistry Laboratory of Fattouma Bouguiba University Hospital, Monastir, Tunisia, using commercial reagent kits. The normal range of serum TSH is 0.15-5 mIU/L and of T4 is 8.6-25 pmol/L. For anti-Tg and anti-TPO, a reciprocal titer of >1:100 was considered positive.

Statistical Analyses. The Hardy-Weinberg equilibrium (HWE) was assessed by the χ^2 test. Codominant and dominant models were used to evaluate the relationship between gene variations and the risk and prognosis of AITD. Alleles and genotypes distribution between patients and controls were estimated using the χ^2 test or Fischer's exact test.

Clinical parameters were compared with *VDR* or *FCGR2A* genotype counts in AITD, HT and GD patients through this separations: age of patients (<40 years old *vs.* ≥40 years old), gender (female *vs.* male), Tg antibody and TPO antibody (positive *vs.* negative). Mean ± and SD were given for T4 and TSH hormone profile for HT and GD patients and a nonparametric test (Mann-Whitney test or Wilcoxon test) was realized to detect a significant difference in hormone levels between genotypes. The Statistical Package for Social Science (SPSS) version 23 (IBM Corp., Armonk, NY, USA), and Epi Info 7 (Center for Disease Control and Prevention, Atlanta, GA, USA) programs enabled statistical analysis. A p value of less than 0.05 was considered statistically significant.

RESULTS

Association of *VDR* Gene Polymorphism with AITD Risk. Genotype and allele frequencies for the *VDR* rs 7975232 in controls, AITD, HT and GD patients are presented in Table 1. Both patient and control groups were in HWE. No significant difference in rs7975232 polymorphism frequencies between patients and controls was detected with codominant and dominant models.

Association of *FCGR2A* Gene Polymorphism with AITD Risk. Table 2 shows the genotype and allele frequencies for the *FCGR2A* gene rs1801274 polymorphism in healthy controls, AITD, HT and GD patients. Both patient and control groups are in HWE. The *FCGR2A* genotypes frequency was more important in AITD and HT patients than in controls. The OR (odds ratio) in the presence of the 131HH genotype were 2.18 and 2.39 in AITD and HT patients, respectively. The dominant model (RH + HH *vs.* RR) showed that individuals with the H alleles were significantly more frequent in AITD (p = 0.03) and HT (p = 0.01) patients than in controls. The frequency of the H allele was also higher in AITD (p = 0.011) and HT (p = 0.012) patients than in controls. However, genotypic and allelic distribution between GD patients and controls was not significantly different (Table 2).

Association of *VDR* Gene Polymorphism with Clinical Characteristics. Genotype and allele frequencies of rs7975232 variation depending on clinical parameters

Table 1. Distribution of *VDR* rs7975232 polymorphism genotypes and alleles in healthy control (n = 162), in autoimmune thyroid disease patients AITD patients (n = 162), Hashimoto's thyroiditis patients (n = 106) and Graves' disease patients (n = 56).

	Controls	AITD Patients			HT Patients			GD Patients		
Genotypes/ Alleles	*n* (%)	*n* (%)	*p* (χ^2)	OR (95% CI)	*n* (%)	*p* (χ^2)	OR (95% CI)	*n* (%)	*p* (χ^2)	OR (95% CI)
CC	58 (35.8)	49 (30.2)		reference	30 (28.3)		reference	19 (33.9)		reference
CA	68 (42.0)	69 (42.6)	0.56 (0.33)	1.20 (0.72-1.99)	49 (46.2)	0.32 (0.97)	1.39 (0.78-2.47)	20 (35.7)	0.91 (0.01)	0.89 (0.43-1.84)
AA	36 (22.2)	44 (27.2)	0.27 (1.20)	1.44 (0.80-2.58)	27 (25.5)	0.35 (0.85)	1.45 (0.74-2.82)	17 (30.4)	0.46 (0.52)	1.44 (0.66-3.12)
CA+AA[a]	104 (64.2)	113 (69.8)	0.34 (0.89)	1.28 (0.80-2.04)	76 (71.7)	0.25 (1.31)	1.41 (0.83-2.40)	37 (66.1)	0.92 (0.008)	1.08 (0.57-2.05)
C	184 (56.8)	167 (51.5)		reference	109 (51.4)		reference	58 (51.8)		reference
A	140 (43.2)	157 (48.5)	0.20 (1.59)	1.23 (0.90-1.68)	103 (48.6)	0.25 (1.28)	1.24 (0.87-1.75)	54 (48.2)	0.41 (0.65)	1.22 (0.79-1.88)

AITD: autoimmune thyroid disease patients; HT: Hashimoto's thyroiditis patients; GD: Graves' disease patients; *n*: number of individuals.
[a] Dominant model.

Table 2. Distribution of *FCGR2A* rs1801274 polymorphism genotypes and alleles in healthy controls (n = 162), autoimmune thyroid disease patients (n = 162), Hashimoto's thyroiditis patients (n = 106) and Graves' disease patients (n = 56).

	Controls	AITD Patients			HT Patients			GD Patients		
Genotypes/ Alleles	*n* (%)	*n* (%)	*p* (χ^2)	OR (95% CI)	*n* (%)	*p* (χ^2)	OR (95% CI)	*n* (%)	*p* (χ^2)	OR (95% CI)
RR	62 (38.3)	43 (26.6)		reference	24 (22.6)		reference	19 (34.0)		reference
RH	73 (45.0)	78 (48.1)	0.11 (2.43)	1.54 (0.93-2.54)	57 (53.8)	0.026 (4.95)	2.01 (1.12-3.62)	21 (37.5)	0.99 (0.00)	0.93 (0.46-1.90)
HH	27 (16.7)	41 (25.3)	0.019 (5.43)	2.18 (1.17-4.07)	25 (23.6)	0.026 (4.91)	2.39 (1.16-4.91)	16 (28.5)	0.15 (1.98)	1.93 (0.86-4.34)
RH+HH[a]	100 (61.7)	119 (73.4)	0.030 (4.56)	1.71 (1.07-2.74)	82 (77.4)	0.01 (6.48)	2.11 (1.21-3.68)	37 (66.0)	0.67 (0.17)	1.20 (0.63-2.28)
R	197 (60.8)	164 (50.6)		reference	105 (49.5)		reference	59 (52.7)		reference
H	127 (39.2)	160 (49.4)	0.011 (6.40)	1.51 (1.10-2.06)	107 (50.5)	0.012 (6.17)	1.58 (1.11-2.24)	53 (47.3)	0.16 (1.94)	1.39 (0.90-2.14)

AITD: autoimmune thyroid disease patients; HT: Hashimoto's thyroiditis patients; GD: Graves' disease patients; *n*: number of individuals.
[a] Dominant model.

of AITD and GD patients are shown in Tables 3 and 4. Significant association was found with the age of AITD and GD patients during the first diagnosis. Indeed, the genotype AA was more common in AITD and GD patients younger than 40 years than those patients older than 40 years with $p = 0.02$ and $p = 0.03$ values, respectively. This result also demonstrated that the A allele was more frequent in AITD and GD patients younger than 40 years of age with $p = 0.01$ and $p = 0.009$ values, respectively.

A significant association was also found between the A allele and the absence or presence of anti-Tg antibodies. The percentage of AITD patients with allele A was higher in the presence (56.7%) than in the absence (39.8%) of anti-Tg antibodies (Table 3). The rs7975232 variation was not associated with gender, age and the presence or absence of Tg and TPO antibodies in HT patients (data not shown). The TSH and T4 levels of GD and HT patients were compared in accordance with *VDR* genotypes (Table 5). The genotype AA was associated with a significant increase of T4 level with regard to those with CC or CA genotypes in HT patients ($p = 0.044$). Nonetheless, TSH and T4 levels in GD patients were not different depending on the *VDR* genotypes.

Association of *FCGR2A* Gene Polymorphism with Clinical Characteristics. Genotype and allele frequencies of the rs1801274 polymorphism in terms of clinical characteristics of HT patients are presented in Table 6. The H allele frequency was lower in women (48.5%) than in men (78.6%). It was associated with gender as a protective factor in women HT patients ($p = 0.04$). The rs1801274 polymorphism was not associated with gender, age and the absence or presence of Tg and TPO antibodies in AITD and GD patients (data not shown). The TSH and T4 levels of GD and HT patients were compared in accordance with *FCGR2A* genotypes (Table 5). The genotype HH was associated with a significant decrease of T4 level with regard to those with RR or RH genotypes in GD patients ($p = 0.015$), although TSH and T4 levels in HT patients were not different depending on the *FCGR2A* genotypes.

Table 3. Distribution of *VDR* rs7975232 polymorphism genotypes and alleles in autoimmune thyroid disease patients according to clinical parameters.

	Gender			Age			Anti-Tg Antibodies			Anti-TPO Antibodies		
Genotypes/ Alleles	F *n* (%)	M *n* (%)	*p*	<40 *n* (%)	≥40 *n* (%)	*p*	[–] *n* (%)	[+] *n* (%)	*p*	[–] *n* (%)	[+] *n* (%)	*p*
CC	46 (32.0)	3 (16.7)		8 (19.0)	41 (34.2)		18 (41.0)	12 (26.7)		13 (34.2)	18 (37.6)	
CA	58 (40.2)	11 (61.1)	NS	17 (40.5)	52 (43.3)	NS	17 (38.6)	15 (33.3)	NS	14 (36.8)	15 (31.2)	NS
AA	40 (27.8)	4 (22.2)	NS	17 (40.5)	27 (22.5)	*p*: 0.02; (χ^2: 4.79) OR: 3.22; 95% CI: 1.22-8.51	9 (20.4)	18 (40.0)	NS	11 (29.0)	15 (31.2)	NS
CA+AA[a]	98 (68.0)	15 (83.3)	NS	34 (81.0)	79 (65.8)	NS	26 (59.0)	33 (73.3)	NS	25 (65.8)	30 (62.5)	NS
C	150 (52.0)	17 (47.2)		33 (39.3)	134 (55.8)		53 (60.2)	39 (43.3)		40 (52.6)	51 (53.1)	
A	138 (48.0)	19 (52.8)	NS	51 (60.7)	106 (44.2)	*p*: 0.01; (χ^2: 6.17) OR: 1.95; 95% CI: 1.17-3.24	35 (39.8)	51 (56.7)	*p*: 0.03; (χ^2: 4.43); OR: 1.98; 95% CI: 1.09-3.59	36 (47.4)	45 (46.9)	NS

AITD: autoimmune thyroid disease patients; Anti-Tg antibodies: anti-thyroglobulin antibodies; Anti-TPO antibodies: anti-thyroid peroxidase antibodies; F: female; M: male; NS: not significant.

[a] Dominant model.

Table 4. Distribution of *VDR* rs7975232 polymorphism genotypes and alleles in Graves' disease patients according to clinical parameters.

	Gender			Age			Anti-Tg Antibodies			Anti-TPO Antibodies		
Genotypes/ Alleles	F *n* (%)	M *n* (%)	*p*	<40 *n* (%)	≥40 *n* (%)	*p*	[–] *n* (%)	[+] *n* (%)	*p*	[–] *n* (%)	[+] *n* (%)	*p*
CC	17 (37.8)	2 (18.2)		3 (20.0)	16 (39.0)		7 (53.9)	3 (21.4)		6 (42.8)	5 (38.5)	
CA	13 (28.9)	7 (63.6)	NS	3 (20.0)	17 (41.5)	NS	4 (30.7)	5 (35.7)	NS	4 (28.6)	5 (38.5)	NS
AA	15 (33.3)	2 (18.2)	NS	9 (60.0)	8 (19.5)	*p*[b]: 0.03; OR: 6.00; 95% CI:1.26-28.49	2 (15.4)	6 (42.9)	NS	4 (28.6)	3 (23.0)	NS
CA+AA[a]	28 (62.2)	9 (81.8)	NS	12 (80.0)	25 (61.0)	NS	6 (46.1)	11 (78.6)	NS	8 (57.2)	8 (61.5)	NS
C	47 (52.2)	11 (50.0)		9 (30.0)	49 (59.8)		18 (69.2)	11 (39.3)		16 (57.1)	15 (57.7)	
A	43 (47.8)	11 (50.0)	NS	21 (70.0)	33 (40.2)	*p*: 0.009; (χ^2: 6.64); OR: 3.46; 95% CI: 1.41-8.49	8 (30.8)	17 (60.7)	*p*: 0.05; (χ^2: 3.73); OR: 3.47; 95% CI: 1.12-10.72	12 (42.9)	11 (42.3)	NS

Anti-Tg antibodies: anti-thyroglobulin antibodies, Anti-TPO antibodies: anti-thyroid peroxydase antibodies, F: female; M: male; NS: not significant.

[a] Dominant model.

[b] Fisher's exact test.

Table 5. Hormone levels among the *VDR* and the *FCGR2A* genotypes in Hashimoto's thyroiditis and Graves' disease patients.

	HT Patients				GD Patients			
VDR rs7975232	TSH[a]	*p*	T4[a]	*p*	TSH[a]	*p*	T4[a]	*p*
CC	56.1±105.8		9.5±7.6		0.10±0.40		50.4±52.6	
CA	47.0±94.5	NS	8.3±4.3	NS	0.60±1.80	NS	38.4±25.2	NS
AA	117.2±176.3	NS	24.5±90.2	0.044	0.10±0.50	NS	43.3±59.2	NS
FCGR2A rs1801274								
RR	110.3±182.9		6.4±4.6		0.01±0.02		63.7±57.2	
RH	68.2±118.9	NS	19.1±65.7	NS	0.40±1.50	NS	41.3±22.1	NS
HH	55.7±86.8	NS	6.9±4.8	NS	0.10±0.50	NS	31.3±23.0	0.015

HT: Hashimotos thyroiditis; GD: Grave's disease; TSH: thyroid stimulating hormone;T4: tetraiodothyroxine, NS: no significant.

[a] The range of normal values for TSH is 0.15-5.0 mIU/L and for T4 is 8.6-25.0 pmol/L.

Table 6. Distribution of *FCGR2A* rs1801274 polymorphism genotypes and alleles in Hashimoto's thyroiditis patients according to clinical parameters.

	Gender			Age			Anti-Tg Antibodies			Anti-TPO Antibodies		
Genotypes/ Alleles	F *n* (%)	M *n* (%)	*p*	<40 *n* (%)	≥40 *n* (%)	*p*	[–] *n* (%)	[+] *n* (%)	*p*	[–] *n* (%)	[+] *n* (%)	*p*
RR	24 (24.3)	0 (0.0)		6 (22.3)	18 (22.8)		6 (19.3)	6 (19.3)		5 (20.8)	6 (17.1)	
RH	54 (54.5)	3 (42.9)	NS	16 (59.2)	41 (51.9)	NS	15 (48.4)	17 (54.9)	NS	12 (50.0)	19 (54.3)	NS
HH	21 (21.2)	4 (57.1)	NS	5 (18.5)	20 (25.3)	NS	10 (32.3)	8 (25.8)	NS	7 (29.2)	10 (28.6)	NS
RH+HH[a]	75 (75.7)	7 (1.0)	NS	21 (77.7)	61 (77.2)	NS	25 (80.7)	25 (80.7)	NS	19 (79.2)	29 (82.9)	NS
R	102 (51.5)	3 (21.4)		28 (51.8)	77 (48.7)		27 (43.5)	29 (46.8)		22 (45.8)	31 (44.3)	
H	96 (48.5)	11 (78.6)	*p*[b]: 0.04; OR: 0.25; 95% CI: 0.06-0.94	26 (48.2)	81 (51.3)	NS	35 (56.5)	33 (53.2)	NS	26 (54.2)	39 (55.7)	NS

Anti-Tg antibodies: anti-thyroglobulin antibodies; Anti-TPO antibodies: anti-thyroid peroxydase antibodies; F: female, M: male, NS: no significant.

[a] Dominant model.

[b] Fisher's exact test.

DISCUSSION

The present study evaluated the association of the rs7975232 *VDR* and rs1801274 *FCGR2A* gene variations with the risk and the prognosis of AITD. The rs7975232 *VDR* polymorphism was not associated with GD and HT. It does not seem to be involved in the susceptibility to AITD in the Tunisian population; this confirms the result of Giovinazzo *et al.* [5], Collins *et al.* [18], and Zarrin *et al.* [19], who showed that there was no association of the rs7975232 *VDR* polymorphism with the AITD in Italian, UK Caucasian and Iranian populations, respectively. This study was in agreement with the results of the several genome wide studies, which have shown that this polymorphism (rs7975232) did not affect the individual susceptibility to AITD [9,20]. In 2019, Maciejewski *et al.* [21] carried out a meta-analysis, which showed that the *VDR* gene is not a very considerable genetic factor in the HT pathogenesis in the Polish Caucasian population [21]. Moreover, Horst-Sikorska *et al.* [22] suggested that the rs7975232 polymorphism was not implicated in susceptibility to GD in the female Polish population. However, Meng *et al.* [9] and Gao *et al.* [10] found a relationship between rs7975232 polymorphism and susceptibility to GD and AITD in Chinese and African populations.

These contradictory results may be due to the different ethnic backgrounds of the populations or to the fact that the studies with a reduced number of individuals do not have significant statistical power to show real associations. The *VDR* rs7975232 (RFLP *Apa*I) polymorphism association with AITD in some populations may also be explained by a linkage disequilibrium with other polymorphisms such as *Bsm*I, *Taq*I and polyA variable number of tandem repeats (VNTR) in the 3'UTR of the *VDR* gene [23,24]. Considering the *FCGR2A* gene, we have demonstrated, for the first time, a significant association between AITD, especially HT pathogenesis and the rs1801274 polymorphism. In fact, people with the H allele are more likely to have AITD (OR = 1.51) or HT (OR = 1.58) than people with the R allele. We demonstrated that the HH genotype could be responsible for approximately a two-fold increased risk of AITD and HT. This result

was confirmed by a dominant model. The H allele carriers (RH + HH) had a higher susceptibility to AITD (OR = 1.71) and to HT (OR = 2.11). These finding may be due to the increased specificity of FcγRIIA H131 variant to IgG2 [15]. Indeed, it has been shown that an effective and prolonged interaction between FcγRIIA and IgG2 can lead to leukocyte activation and chronic inflammation, which could stimulate autoimmunity and tissue destruction [25].

On the other hand, the FcγRIIA-IgG complex leads to transcriptional activation of proinflammatory cytokine genes involved in the pathogenesis of AITD and essentially HT [13]. Effectively, HT is known to be a Th1-dominant disease. It has been shown that certain proinflammatory cytokines produced by Th1 cells, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), IL-1β and tumor necrosis factor (TNF) activate the destruction of thyrocytes [26,27]. Our findings propose that the rs1801274 variation may be a susceptibility marker for HT and AITD but not for GD. To the best of our knowledge, association between rs1801274 polymorphism and HT has never been explored in our population. However, only one study has shown that this genetic variation predisposes to GD [28]. Our analysis findings, depending on age and gender of patients, indicated that solely *FCGR2A* gene variation change the etiology of HT between men and women, also only *VDR* variation increases AITD and GD risk in patients younger than 40 years. Moreover, we analyzed the association of the *VDR* rs7975232 and *FCGR2A* rs1801274 polymorphisms with the severity and the prognosis of AITD, HT and GD. Our findings did not show an association between the *FCGR2A* rs1801274 variation and the presence of anti-Tg and anti-TPO antibodies in all patients. These results imply that the humoral immune response in the thyroid would not depend on *FCGR2A* variation. However, we have found a relevant result, which proves that the rs1801274 variation affects the T4 hormone concentration only in GD patients. Indeed the genotype 131HH was linked to low T4 levels in GD patients and so it could protect against the increase of T4 concentration at the onset of GD.

The originality of this study is that we have shown that the rs7975232 A allele increases the risk of producing anti-Tg antibodies at the onset of disease in AITD patients. Therefore, the *VDR* rs7975232 polymorphism is a genetic marker of Tg-antibodies positive AITD related to progression of disease. The A allele can play a role in the development of AITD through the regulation of anti-Tg antibodies production. Recently, Choi *et al.* [29] have shown that variations in TPO and Tg antibodies titers could be linked to GD relapse after antithyroid drug therapy [29]. Moreover, our results suggested that the *VDR* rs7975232 polymorphism protects against the decrease of T4 level in HT patients at the onset of disease. Indeed, carriers of the AA had a level of T4 that tends toward the normal value.

In conclusion, this investigation indicates that only *FCGR2A* rs1801274 polymorphism could be related to the risk of AITD and HT and could change the etiology of HT between males and females. Moreover, the *VDR* polymorphism increases the risk of early onset of AITD and GD. Furthermore, *VDR* rs7975232 and *FCGR2A* rs1801274 gene polymorphisms could be a prognostic factor for predicting the severity of AITD, HT and GD. Considering the inconsistent findings of *VDR* gene polymorphisms in various ethnic populations, additional investigations, with larger numbers of patients and controls, are required to evaluate the involvement of the *VDR* rs7975232 polymorphism in the predisposition to AITD. Further studies, notably a functional analysis of *VDR* rs7975232 and *FCGR2A* rs1801274 polymorphisms, are requisite to explain the accurate action of these variations on AITD in the Tunisian population. Additional association studies and a haplotypic analysis of several polymorphisms in the Tunisian population are also necessary. These studies will make it possible to carry out a test, which will have an important predictive value for AITD, by associating several genetic variants and by integrating biological data of these diseases.

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