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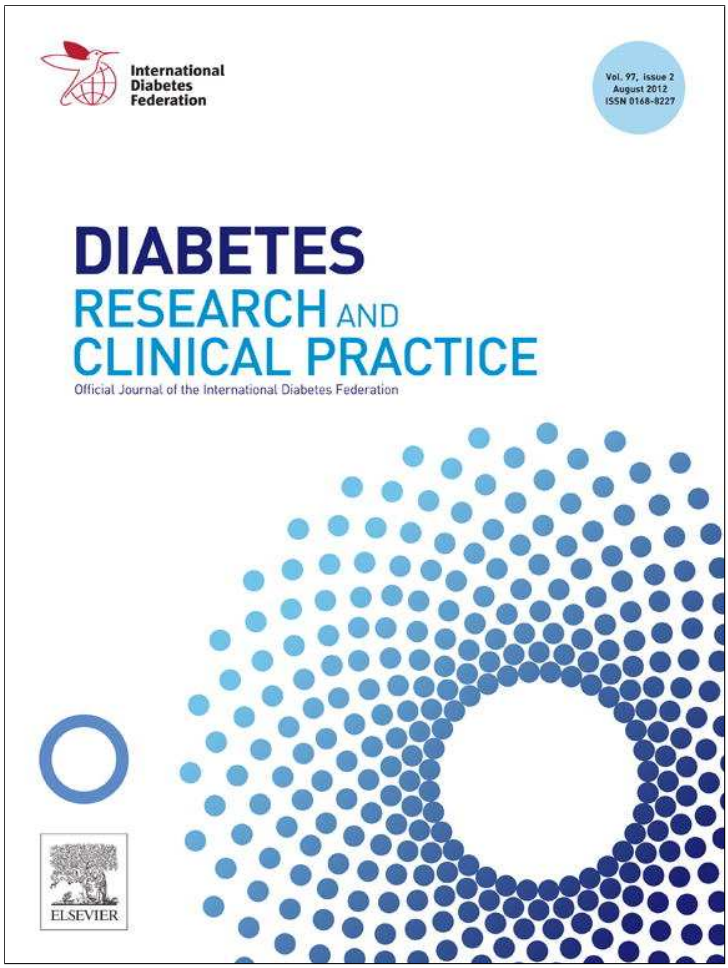
Diabetes Research and Clinical Practice



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Single-nucleotide polymorphisms and haplotypes in the adiponectin gene contribute to the genetic risk for type 2 diabetes in Tunisian Arabs

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ABSTRACT

Adiponectin is an adipocyte-produced protein involved in regulating glucose, lipid, and energy metabolism, and is encoded by *ADIPOQ* (*APM1*) gene. *ADIPOQ* polymorphisms were previously associated with type 2 diabetes (T2DM) in Caucasian and non-Caucasian populations. We investigated the contribution of 13 polymorphisms in the promoter, coding regions, and 3′untranslated region of *ADIPOQ* gene to T2DM in 917 patients and 748 normoglycemic control subjects. *ADIPOQ* genotyping was done by allelic discrimination method. Of the 13 *ADIPOQ* variants analyzed, higher minor allele frequency of rs16861194 ($P < 0.001$), rs17300539 ($P < 0.001$), rs266729 ($P < 0.001$), rs822396 ($P = 0.02$), rs2241767 ($P = 0.03$), and rs1063538 ($P = 0.02$) were seen in T2DM cases. Varied association of *ADIPOQ* genotypes with T2DM was seen according to the genetic model used: rs17300539 and rs266729 were significantly associated with T2DM under the three models, while rs16861194 was association with T2DM under additive and dominant models, and rs822396, rs2241766, and rs1063538 were associated with T2DM under the dominant models only. Haploview analysis revealed low linkage disequilibrium between the *ADIPOQ* variants, resulting in high haplotype diversity, and two blocks were identified, each differentially associated with T2DM. These results support a significant association of *ADIPOQ* gene polymorphism with T2DM in Tunisian Arabs.

1. Introduction

Adiponectin is a 30 kDa adipocyte-secreted hormone, involved in the regulation of blood glucose levels, insulin sensitivity, and lipid metabolism [1,2]. It is an abundant plasma protein constituting 0.01% of total plasma protein, and circulates at 3 and 30 μg/ml concentrations [3,4]. Variability in adiponectin levels was reported according to age, gender (higher in females), and body mass [5–7]. In contrast to other adipocyte-secreted factors, adiponectin levels are markedly low in obese individuals, but increase upon weight reduction [6,8], and in type 2 diabetes (T2DM) patients compared to normoglycemic control subjects [3,9], and are correlated with the level of insulin sensitivity and insulinemia [8,10]. These observations suggested that low plasma adiponectin might contribute to the pathogenesis of insulin resistance and T2DM. As such, several groups have proposed that the *ADIPOQ* gene,

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which encodes adiponectin, is a candidate T2DM susceptibility gene [3,4,11].

Adiponectin is encoded by the *ADIPOQ* (*APM1*) gene (adipose most abundant gene transcript 1), which maps to chromosome 3q27 and consists of three exons and two introns spanning 17 kb [4,12], and a total of 149 SNPs in the *ADIPOQ* gene were identified. These comprised 13 5′ near gene, 16 missense, 6 synonymous, 33 3′UTR, and 81 intronic SNPs. The strong linkage of this genomic region to insulin resistance and T2DM suggested that *ADIPOQ* is a candidate gene for T2DM, metabolic syndrome, and related diseases [4,13,14]. Several variants in the *ADIPOQ* gene were associated with T2DM [11,14–16], obesity [16,17], and the metabolic syndrome [3,4] both in Caucasian and non-Caucasian populations. These include +45T/G, +276G/T, and −3971A/G, which were linked with T2DM in Asians [15,18,19], and the promoter −11426A/G, −11391G/A, and −11377 SNPs, which were associated with T2DM in European Caucasians [11,14,18,20,21]. This qualified the *ADIPOQ* gene as a T2DM candidate susceptibility gene.

Inconsistency in the association of *ADIPOQ* SNPs with T2DM was reported for various populations, and the association of specific *ADIPOQ* SNPs with T2DM was replicated in some, but not all studies [20,22,23]. For example, a significant association of the +45T/G variant with T2DM was reported for Japanese [22], and Chinese [19], while a study on Iranian [24] and Polish [21] subjects found no such association. Furthermore, the +276G/T variant was associated with T2DM in Polish [21] and Japanese [22], but not in Iranians [24] or Chinese [15] subjects. No systematic analysis of the *ADIPOQ* gene with regard to T2DM was previously reported for Arab population. In this study, we examined the association of 13 common variants in the coding, promoter, and 3′untranslated region (UTR) of *ADIPOQ* gene in North African Tunisian subjects of Arabic descent. The contribution of these variants to T2DM was analyzed at the allele, genotype, and haplotype levels.

2. Subjects and methods

2.1. Subjects

The study group included 917 (495 females, 422 males) consecutive unrelated T2DM patients, who attended the outpatient diabetes clinics at Farhat Hached Hospital in Sousse and Fattouma Bourguiba Hospital in Monastir, Tunisia. T2DM diagnosis was based on clinical and laboratory criteria, as per the 1998 WHO diagnostic and classification criteria. None of the patients had ever had ketoacidosis, and T2DM treatment included oral anti-diabetic drugs and/or insulin; all subjects commenced on insulin therapy had been treated with oral drugs for at least two years (Table 1). The control group included 748 unrelated healthy volunteers (375 females and 373 males) with no known personal or family history of diabetes, and from the same geographical area as the patients (Central Tunisia). None of the controls was first-degree relatives of other subjects in the control or study groups; they were not known to have diabetes although occult disease was not excluded. As the genetic origin of the inhabitants of Central Tunisia where the study was conducted is mainly Arab, all participants were Tunisian Arabs; non-Arab Tunisian Berbers (descendants of ancient Vandals) and other minorities were excluded. The University of Monastir Ethics Committee approved the study, which was done according to Helsinki guidelines, and informed consent was obtained from all participants.

Demographic details were recorded on all subjects. These included age, gender, ethnic origin, age of onset, duration and first-degree family history of diabetes, history of hypertension, dyslipidaemia, ischaemic heart disease and other medical illness. In addition, history of chronic diabetes complications, treatment for diabetes (including date of initiation and/or

Table 1 – Clinical characteristic of patients and controls.

Characteristic	Controls (748)	Patients (917)	*P* value
Gender (M/F)	373:375	422:495	0.126[a]
Age at examination (years)	58.7 ± 8.7	59.3 ± 10.9	0.169[b]
Mean BMI (kg/m^2)	23.5 ± 2.2	27.7 ± 4.3	<0.001[b]
Waist–hip ratio	0.84 ± 0.08	0.93 ± 0.09	<0.001[b]
Family history of diabetes; *n* (%)	0 (0.0)	330 (36.0)	N/A[c]
Diabetes duration (years)	N/A	12.6 ± 6.3	N/A
Age of onset (years)	N/A	46.7 ± 10.9	N/A
Hypertension; *n* (%)	86 (18.0)	420 (45.8)	<0.001[a]
SBP (mmHg)	121.6 ± 14.4	140.7 ± 27.0	<0.001[b]
DBP (mmHg)	77.9 ± 10.5	81.9 ± 12.6	<0.001[b]
Glucose (mmol/L)	5.1 ± 0.6	12.7 ± 5.3	<0.001[b]
HbA1c (%)	4.5 ± 1.4	9.6 ± 3.9	<0.001[b]
Urea (mmol/L)	5.6 ± 2.1	7.9 ± 4.8	<0.001[b]
Creatinine (mmol/L)	63.1 ± 27.3	99.2 ± 35.6	<0.001[b]
HDL (mmol/L)	1.2 ± 0.4	1.0 ± 0.3	<0.001[b]
LDL (mmol/L)	2.8 ± 1.8	3.8 ± 1.4	<0.001[b]
Total cholesterol (mmol/L)	4.6 ± 1.2	5.3 ± 1.4	<0.001[b]
Triglycerides (mmol/L)	1.2 ± 0.6	1.8 ± 1.3	<0.001[b]

[a] Pearson's chi square test.
[b] Student's *t*-test.
[c] N/A = not applicable.

discontinuation of oral agents or insulin) was recorded; the historical information was verified from the clinic records. After an overnight fast, venous blood samples were collected for biochemical analysis, and for genomic DNA extraction.

2.2. *ADIPOQ genotyping*

We screened *ADIPOQ* gene polymorphisms, using SNPbrowser 4.0 (Applied Biosystems, Foster City, CA, USA). *ADIPOQ* gene contains 149 SNPs (13, 5′ near gene; 16, mis-sense; 6, synonymous; 33, 3′UTR; and 81, introns), and the 13 SNPs studied were from the promoter, intron, exons, and 3′UTR region of *ADIPOQ* gene spanning 13 kb of the 17 kb *ADIPOQ* gene, and were selected from the HapMap Caucasian database (CEU) to with minor allele frequency (MAF) >0.02, and based on previous association with T2DM. Pairwise tag SNPs were selected. Of these, nine SNPs were genotyped by allelic discrimination using TaqMan SNP genotyping assays kit, by individuals unaware of the sample type (patient or control). This relied on inclusion of VIC- and FAM-labeled oligonucleotide primers specific for allele 1 (major allele) and allele 2 (minor allele). TaqMan assays, as assay-on-demand, were ordered from Applied Biosystems: C_33187774_10 (rs17300539), C_2412786_10 (rs266729), C_2910317_10 (rs822395), C_2910316_10 (rs822396), C_26426077_10 (rs2241766), C_7497299_10 (rs1501299), C_2641767_10 (rs2241767), C_27479710_10 (rs3774261), and C_33187743_10 (rs17366743). The reaction was performed in 6 μl volume on ABI7900 system, according to manufacturer's instructions (Applied Biosystems). The remaining four SNPs were genotyped by PCR-based assays due to the unavailability of TaqMan primer sets. Both rs16861194 and rs1063537 were genotyped by allele specific amplification (PCR-ASA), while rs266730 (*Bfa* I) and rs1063538 (*Nsp* I) were genotyped by restriction fragment length polymorphism (PCR-RFLP) analysis, using the indicated restriction endonucleases. Replicate blinded quality control samples were included to assess reproducibility of the genotyping procedure; concordance was >99%.

2.3. *Statistical analysis*

Statistical analysis was performed on SPSS v. 17.0 software (SPSS Inc., Chicago, IL). Data were expressed as mean ± SD for continuous variables, which were normally distributed, or as percentages of total for categorical variables. Pearson χ^2 or Fisher's exact test were used to assess inter-group significance, and Student's *t*-test was used to determine differences in means. Allele frequencies were calculated by the gene-counting method, and each polymorphism was tested for Hardy–Weinberg equilibrium by χ^2 goodness-of-fit test using HPlus 2.5 software (http://qge.fhcrc.org/hplus). Differences in allele and genotype frequencies of *ADIPOQ* variants were tested by Pearson's χ^2 test and Fisher's exact test. After the power was computed for each SNP (http://pngu.mgh.harvard.edu/_purcell), the overall power was calculated as the average power over the SNPs genotyped. At $\alpha = 0.05$, this sample size provided 73.7% power in detecting T2DM susceptibility, assuming a 100% call rate. The association of *ADIPOQ* genotypes with T2DM was conducted under additive, dominant and recessive models, using SNPstats (http://bioinfo.iconcologia.net/SNPstats). Multiple-test correction was performed by the Bonferroni method as per: $Pc = 1 - (1 - P)^n$, where n = number of comparisons. Linkage disequilibrium (LD) analysis and haplotypes reconstruction was done using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview). Haplotype analysis was conducted under no-interaction null hypothesis (multiplicative scale), which assumes that T2DM is not associated with any haplotypes. *ADIPOQ* haplotypes were coded as per the allele at each locus. All *P*-values were two-tailed; *P*-values <0.05 were considered statistically significant.

3. Results

3.1. *Study subjects*

The clinical characteristics of study subjects are reported in Table 1. While gender and age at examination were comparable between cases and controls, significant differences between were noted in mean BMI ($P < 0.001$), systolic and diastolic blood pressure readings and the prevalence of hypertension ($P < 0.001$), serum urea ($P < 0.001$) and creatinine ($P < 0.001$), and serum lipids (HDL, LDLD, total cholesterol, and triglycerides) ($P < 0.001$). Accordingly, the latter were the covariates that were controlled for in subsequent analysis.

3.2. *Association studies*

All 13 *ADIPOQ* SNPs tested were in Hardy–Weinberg equilibrium among control subjects (Table 2). Table 3 summarizes the association between *ADIPOQ* SNPs and T2DM in case-control subjects. Significant differences in the minor allele frequency (MAF) of rs16861194 ($P < 0.001$), rs17300539 ($P < 0.001$), rs266729 ($P < 0.001$), rs822396 ($P = 0.02$), rs2241767 ($P = 0.03$), and rs1063538 ($P = 0.02$) were seen between T2DM cases and control subjects. MAF of the remaining *ADIPOQ* SNPs were comparable between cases and controls.

Table 4 summarizes the results of association between rs16861194, rs17300539, rs266729, rs822396, rs2241767, and rs1063538 *ADIPOQ* variants and T2DM, under additive, dominant and recessive genetic models, after adjustment for the covariates for BMI, gender, hypertension, and serum lipid profile. Both rs17300539 and rs266729 showed a significant

Table 2 – *APM1* SNPs analyzed.

SNP	rs number	Genome position	Gene position	Alleles	*HWE P*
1	rs266730	186558461	−12128	G:A	0.132
2	rs16861194	186559175	−11426	A:G	0.237
3	rs17300539	186559210	−11391	G:A	0.971
4	rs266729	186559224	−11377	C:G	1.000
5	rs822395	186566557	−4041	A:C	0.674
6	rs822396	186566627	−3964	A:G	0.370
7	rs2241766	186570642	45	T:G	0.133
8	rs1501299	186570873	276	G:T	0.634
9	rs2241767	186570946	349	A:G	0.882
10	rs3774261	186571309	712	A:G	0.672
11	rs17366743	186571839	1233	C:T	1.000
12	rs1063537	186573825	3228	C:T	0.880
13	rs1063538	186573933	3286	T:C	0.372

Table 3 – Association of *ADIPOQ* SNPs with T2DM.[a]

rs number	Cases MAF	Controls MAF	χ^2	P[b]	aOR (95% CI)[c]
rs266730	480 (0.26)	375 (0.25)	0.011	0.915	1.05 (0.90–1.23)
rs16861194	227 (0.12)	120 (0.08)	16.28	5.5×10^{-5}	1.65 (1.30–2.09)
rs17300539	230 (0.13)	122 (0.08)	16.30	5.4×10^{-5}	1.62 (1.28–2.04)
rs266729	583 (0.32)	379 (0.25)	16.39	5.1×10^{-5}	1.37 (1.18–1.60)
rs822395	621 (0.34)	468 (0.31)	2.37	0.12	1.12 (0.97–1.29)
rs822396	335 (0.18)	227 (0.15)	5.40	0.02	1.24 (1.03–1.49)
rs2241766	218 (0.12)	174 (0.12)	0.03	0.86	1.02 (0.83–1.26)
rs1501299	554 (0.30)	410 (0.27)	3.01	0.08	1.14 (0.98–1.33)
rs2241767	482 (0.26)	345 (0.23)	4.41	0.03	1.19 (1.02–1.40)
rs3774261	635 (0.35)	477 (0.32)	2.66	0.10	1.12 (0.97–1.30)
rs17366743	30 (0.02)	11 (0.01)	2.08	0.15	1.79 (0.87–3.66)
rs1063537	427 (0.23)	345 (0.23)	0.01	0.91	1.02 (0.87–1.27)
rs1063538	535 (0.41)	535 (0.36)	5.14	0.02	1.18 (1.03–1.36)

MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.
[a] Study subjects (case/control) were: Lebanese (751/918), and Tunisians (1470/838).
[b] Crude P value.
[c] aOR = adjusted odds ratio, adjusted for BMI, gender, hypertension, and serum lipid profile.

Table 4 – T2DM association for candidate SNPs in the Tunisian study sample of 1665 individuals.

SNP	Genotype	Controls: Cases	Additive		Dominant		Recessive	
			P	aOR (95% CI)[a]	P	aOR (95% CI)	P	aOR (95% CI)
rs16861194	A/A	633:698		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)
	A/G	110:211	<0.001	1.74 (1.35–2.24)	<0.001	1.73 (1.34–2.22)	0.620	1.32 (0.43–4.07)
	G/G	5:8		1.47 (0.48–4.52)		[A/G + G/G vs. A/A]		[A/A + A/G vs. G/G]
rs17300539	G/G	630:702						
	G/A	114:200	<0.001	1.57 (1.22–2.03)	<0.001	1.64 (1.27–2.10)	0.029	3.09 (1.02–9.35)
	A/A	4:15		3.37 (1.11–10.19)		[G/A + A/A vs. G/G]		[G/G + G/A vs. A/A]
rs266729	C/C	419:423						
	C/G	279:405	<0.001	1.44 (1.17–1.76)	<0.001	1.49 (1.22–1.81)	0.025	1.50 (1.05–2.15)
	G/G	50:89		1.76 (1.22–2.56)		[C/G + G/G vs. C/C]		[C/C + C/G vs. G/G]
rs822396	A/A	352:406						
	A/G	324:401	0.061	1.27 (1.02–1.58)	0.020	1.28 (1.04–1.59)	0.280	1.36 (0.77–2.39)
	G/G	72:110		1.45 (0.82–2.56)		[A/G + G/G vs. A/A]		[A/A + A/G vs. G/G]
rs2241767	A/A	442:500						
	A/G	267:352	0.097	1.17 (0.95–1.43)	0.061	1.20 (0.99–1.46)	0.110	1.39 (0.92–2.09)
	G/G	39:65		1.47 (0.97–2.24)		[A/G + G/G vs. A/A]		[A/A + A/G + G/G]
rs1063538	T/T	310:331						
	T/C	334:436	0.066	1.22 (0.99–1.51)	0.026	1.25 (1.03–1.53)	0.160	1.21 (0.92–1.59)
	C/C	104:150		1.35 (1.01–1.81)		[T/C + C/C vs. T/T]		[T/T + T/C vs. C/C]

[a] aOR = adjusted odds ratio, adjusted for age, gender, and BMI.

association with T2DM under the three models tested. The association of rs16861194 remained significant under the additive and dominant models only, while rs822396 and rs1063538 were associated with T2DM under the dominant genetic model only. In contrast rs2241767 did not associate significantly with T2DM under any of the genetic models tested, but showed lower magnitude of effect and in the same direction, after adjusting for covariates under the dominant model (Table 4).

3.3. *ADIPOQ haplotypes*

Haploview analysis demonstrated limited linkage disequilibrium (LD) among the *ADIPOQ* SNPs studied (Fig. 1). Based on LD pattern, two blocks were identified: the first (Block 1) containing three SNPs (rs16861194, rs17300539, rs266729), while the second (Block 2) contained four SNPs (rs2241766, rs1501299, rs2241767, and rs3774261). For haplotype analysis, major alleles were coded as ''1'', while minor alleles were coded as ''2''. Within Block 1, reduced frequency of haplotype 111 ($P < 0.001$), and increased frequency of haplotypes 222 ($P = 0.013$), and 212 ($P = 0.005$) was seen in patients than control subjects, thereby assigning disease protective and susceptible nature to these haplotypes, respectively (Table 5). Within Block 2, reduced frequency of haplotypes 1212 ($P = 0.017$), 1222 ($P = 0.032$), 2121 ($P < 0.001$) and 2111 ($P < 0.001$) haplotypes, and increased frequency of haplotype 2221 ($P < 0.001$) was seen in cases than in controls, thereby assigning a protective and susceptible nature to these rs3774261 G allele-containing haplotypes (Table 5). This association remained significant

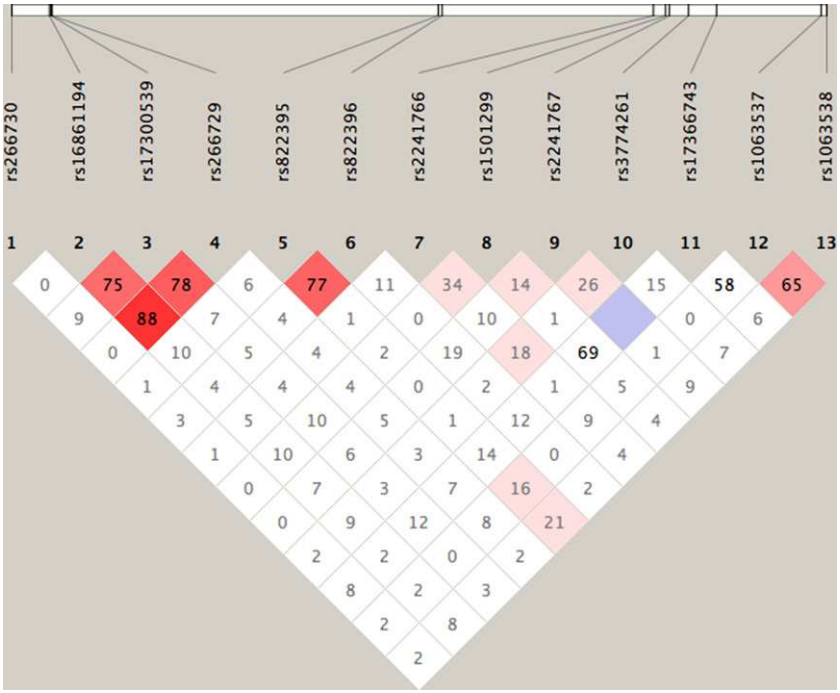

Fig. 1 – Haploview graph of *ADIPOQ* SNPs analyzed; block 1 comprising rs1681194/rs17300539/rs266729, while block 2 consisted of rs2241766/rs1501299/rs2241767/rs3774261. Light red/pink block, D' (normalized linkage disequilibrium measure or D) < 1.0, with logarithm of odds (LOD) score >2.0; white blocks, D' < 1.0 with LOD < 2.0; numbers in blocks denoting D' value. The genomic organization (Build 37.3) is depicted above the LD plot. LOD being defined as $\log_{10}$(L1/L0), where L1 = likelihood of the data under linkage disequilibrium, and L0 = likelihood of the data under linkage equilibrium. D' is calculated as per: D' = (D) divided by the theoretical maximum for the observed allele frequencies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

after controlling for BMI, gender, hypertension, and serum lipid profile.

4. Discussion

Genome wide association studies identified *ADIPOQ* as a T2DM susceptibility locus [25]. In this study, we investigated the association between *ADIPOQ* polymorphisms and T2DM in a large sample of Tunisian Arabs. We included SNPs in the promoter (rs266730, rs16861194, rs17300539, and rs266729) introns (rs822395, rs822396, rs1501299, rs2241767, and rs3774261), exons (rs2241766, and rs17366743), and 3′UTR (rs1063537 and rs1063538) regions of *ADIPOQ*. Previous studies of the association between *ADIPOQ* variants and T2DM examined European or Asian populations [11,21,22,26], and

Table 5 – Haplotype frequencies across 13 *APM1* SNPs analyzed.

Block[a]	Haplotype[b]	Frequency	Case:Control frequencies	χ^2	P
Block 1	**1 1 1**	**0.691**	**0.656; 0.733**	**22.812**	**1.8×10^{-6}**
	1 1 2	0.181	0.189; 0.172	1.66	0.198
	2 2 2	**0.078**	**0.088; 0.065**	**6.21**	**0.013**
	2 1 2	**0.018**	**0.024; 0.011**	**7.75**	**0.005**
Block 2	1 1 1 2	0.329	0.357, 0.283	3.18	0.075
	1 1 1 1	0.181	0.189; 0.174	0.73	0.392
	1 1 2 2	0.119	0.115; 0.130	1.13	0.288
	1 2 1 2	**0.109**	**0.094; 0.142**	**5.74**	**0.017**
	1 2 1 1	0.055	0.047, 0.055	0.10	0.753
	1 2 2 2	**0.046**	**0.026, 0.064**	**4.62**	**0.032**
	2 1 2 1	**0.025**	**0.010, 0.043**	**36.79**	**1.3×10^{-9}**
	2 1 1 1	**0.022**	**0.008, 0.040**	**36.79**	**1.3×10^{-9}**
	2 2 2 1	**0.020**	**0.032, 0.003**	**38.03**	**7.0×10^{-10}**

Boldface indicates significance.
[a] *APM1* block 1: rs1681194/rs17300539/rs266729, block 2: rs2241766/rs1501299/rs2241767/rs3774261haplotypes.
[b] Alleles were coded as "11" (*major allele*) and "2" (*minor allele*).

few studies on populations of African descent (South Africans, African-Americans) [27,28], and no study has assessed the contribution of *ADIPOQ* variants to T2DM in an Arab population. To our knowledge, this is the first study that addressed the association of *ADIPOQ* polymorphisms with T2DM in North-African Tunisian Arab population. The results support the notion that genetic variation in *ADIPOQ* influences T2DM development.

The minor allele frequencies of the *ADIPOQ* SNPs analyzed were generally comparable to the frequencies established for European/Caucasian population (www.hapmap.org). Of the 13 *ADIPOQ* variants studied, significant associations with T2DM risk were noted with −11426A/G (rs16861194), −1139G/A (rs17300539), −11377C/G (rs266729), and to lesser extent −3964A/G (rs822396), +349A/G (rs2241767), and +3286T/C (rs1063538) variants. SNPs rs16861194, rs17300539, rs266729 and rs822396 lie in the promoter region, and rs1063538 is in the 3′-UTR region of *ADIPOQ*, and thus may affect transcription of *ADIPOQ*, as was shown elsewhere [11,29]. Of the promoter SNPs analyzed, we observed association between rs822396 and T2DM in Tunisians. This variant was not previously associated with T2DM or its complications in other populations, although it has been associated with prostate cancer [30] and cerebrovascular disease [31]. While we did not correlate the studied variants with changes in serum adiponectin concentrations in cases and control subjects in our study, it would be of interest to assess whether the contribution of rs822396 and other positive variants to increased T2DM risk is by modulation of plasma adiponectin concentrations.

Varied association of the *ADIPOQ* promoter SNPs with T2DM was previously reported, and an ethnic contribution of this association was evident. In this study, we documented strong association between −11426A/G (rs16861194) and T2DM, in agreement with studies on Caucasian [14,32] and non-Caucasian [33] populations. While −1139G/A (rs17300539) is significantly associated with T2DM in Tunisians, it was not linked with increased T2DM risk in populations of diverse ethnicities [14,20,21,26,32,34]. In addition, the −11377C/G (rs266729) variant was associated with T2DM according to some [26,32,35], but not other [14,34] studies. Furthermore, rs2241766 (T45G) variant was associated with T2DM in Tunisians, Finnish [16], and Italian [34] populations, but not in Asians [26,32], thereby prompting the speculation that rs2241766 is a Caucasian-selective T2DM susceptibility variant. That the association in our Tunisian population collection is at a different SNP is not surprising, given the documented ethnic and geographical differences in adiponectin gene structure [28], and thus the overall association with increased T2DM risk [20,22,23].

Interestingly, the intronic SNP rs2241767, associated with significant increases in coronary artery calcification [36], was markedly associated with T2DM among Tunisians, and also Chinese Han population [37]. While it is not the scope of the current study, it is tempting to speculate that +349A/G (rs2241767) may affect *ADIPOQ* gene expression by repressing translation, or alternatively by inducing alternate cleavage of RNA transcripts, as was suggested elsewhere [38]. This emphasizes the potential of other variants in *ADIPOQ* gene linked with T2DM risk.

Hapolview analysis demonstrated weak LD between the *ADIPOQ* SNPs analyzed, in agreement with recent studies on US African-American and White [29], Chinese [19], and French Caucasian [22] populations. The differences in haplotype assignments between Tunisians in this study and Caucasian and non-Caucasian populations is likely attributed to differential LD patterns within race, as was suggested [29]. Confirmatory studies are needed to validate, or alternatively rule out, the associations between specific *ADIPOQ* haplotypes with increased T2DM risk.

Compared with previous studies, our study has some important strength. First, a large number of subjects were included (917 T2DM patients and 748 controls), which was sufficiently powered to reduce type I errors. Second, the study was performed in an ethnically homogeneous group of individuals (North African Tunisian Arabs), which increase the validity of the statistical analysis. Third, we controlled for several covariates in the association studies, in particular BMI, lipid profile, and hypertension, which were modified by specific *ADIPOQ* genotpyes [11,36]. However, our study has some limitations, namely that we did not measure serum adiponectin levels, and thus could not perform genotype-phenotype correlation studies, and that it was limited to a specific ethnic group (North African Tunisian Arabs), thereby necessitating follow-up studies from different ethnicities. These, coupled with the potential linkage of *ADIPOQ* polymorphisms studied with other *ADIPOQ* or nearby gene polymorphisms, points to the need for future large population-based case-control studies, especially those stratified for gene-environment interaction.

Author contributions

N.M.: Performed genotyping assays, and researched data; I.E.: Prepared specimens, performed genotyping assays; A.T.: Prepared specimens, performed genotyping assays; A.C.: Screened cases, referred cases to study; T.M.: Researched data and contributed to discussion; W.A.: Analyzed the data, wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003;4:459–69.
[2] Ziemke F, Mantzoros CS. Adiponectin in insulin resistance: lessons from translational research. Am J Clin Nutr 2010;91:258S–61S.
[3] Gable DR, Hurel SJ, Humphries SE. Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic

syndrome and cardiovascular disease. Atherosclerosis 2006;188:231–44.
[4] Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes 2006;55:375–84.
[5] Côté M, Cartier A, Reuwer AQ, Arsenault BJ, Lemieux I, Després JP, et al. Adiponectin and risk of coronary heart disease in apparently healthy men and women (from the EPIC-Norfolk Prospective Population Study). Am J Cardiol 2011;108:367–73.
[6] Lang HF, Chou CY, Sheu WH, Lin JY. Weight loss increased serum adiponectin but decreased lipid levels in obese subjects whose body mass index was lower than 30 kg/m^2. Nutr Res 2011;31:378–86.
[7] Liu J, Wang F, Cha Y, Chen ZP, Ding HY. Adiponectin levels in non-obese first-degree relatives of type 2 diabetes patients and non-diabetic subjects: a 5-year follow-up study. J Int Med Res 2010;38:792–802.
[8] Pyrzak B, Ruminska M, Popko K, Demkow U. Adiponectin as a biomarker of the metabolic syndrome in children and adolescents. Eur J Med Res 2010;15(Suppl 2):147–51.
[9] Medina-Bravo P, Meza-Santibáñez R, Rosas-Fernández P, Galván-Duarte R, Saucedo-García R, Velázquez-López L, et al. Decrease in serum adiponectin levels associated with visceral fat accumulation independent of pubertal stage in children and adolescents. Arch Med Res 2011;42:115–21.
[10] Hung J, McQuillan BM, Thompson PL, Beilby JP. Circulating adiponectin levels associate with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity. Int J Obes (Lond) 2008;32:772–9.
[11] Hivert MF, Manning AK, McAteer JB, Florez JC, Dupuis J, Fox CS, et al. Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes 2008;57:3353–9.
[12] Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M, et al. Genomic structure and mutations in adipose-specific gene, adiponectin. Int J Obes Relat Metab Disord 2000;24:861–8.
[13] Francke S, Manraj M, Lacquemant C, Lecoeur C, Leprêtre F, Passa P, et al. A genome-wide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. Hum Mol Genet 2001; 10:2751–65.
[14] Gibson F, Froguel P. Genetics of the APM1 locus and its contribution to type 2 diabetes susceptibility in French Caucasians. Diabetes 2004;53:2977–83.
[15] Li Y, Li X, Shi L, Yang M, Yang Y, Tao W, et al. Association of adiponectin SNP + 45 and SNP + 276 with type 2 diabetes in Han Chinese populations: a meta-analysis of 26 case-control studies. PLoS One 2011;6:e19686.
[16] Siitonen N, Pulkkinen L, Lindström J, Kolehmainen M, Eriksson JG, Venojärvi M, et al. Association of ADIPOQ gene variants with body weight, type 2 diabetes and serum adiponectin concentrations: the Finnish Diabetes Prevention Study. BMC Med Genet 2011;12:5.
[17] Park JW, Park J, Jee SH. ADIPOQ gene variants associated with susceptibility to obesity and low serum adiponectin levels in healthy Koreans. Epidemiol Health 2011;33:e2011003.
[18] Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 2002;11:2607–14.
[19] Tso AW, Sham PC, Wat NM, Xu A, Cheung BM, Rong R, et al. Polymorphisms of the gene encoding adiponectin and glycaemic outcome of Chinese subjects with impaired glucose tolerance: a 5-year follow-up study. Diabetologia 2006;49:1806–15.
[20] Gong M, Long J, Liu Q, Deng HC. Association of the ADIPOQ rs17360539 and rs266729 polymorphisms with type 2 diabetes: a meta-analysis. Mol Cell Endocrinol 2010; 325:78–83.
[21] Szopa M, Malczewska-Malec M, Kiec-Wilk B, Skupien J, Wolkow P, Malecki MT, et al. Variants of the adiponectin gene and type 2 diabetes in a Polish population. Acta Diabetol 2009;46:317–22.
[22] Vasseur F, Leprêtre F, Lacquemant C, Froguel P. The genetics of adiponectin. Curr Diab Rep 2003;3:151–8.
[23] Vionnet N, Tregouët D, Kazeem G, Gut I, Groop PH, Tarnow L, et al. Analysis of 14 candidate genes for diabetic nephropathy on chromosome 3q in European populations: strongest evidence for association with a variant in the promoter region of the adiponectin gene. Diabetes 2006;55:3166–74.
[24] Mohammadzadeh G, Zarghami N. Associations between single-nucleotide polymorphisms of the adiponectin gene, serum adiponectin levels and increased risk of type 2 diabetes mellitus in Iranian obese individuals. Scand J Clin Lab Invest 2009;69:764–71.
[25] Richards JB, Waterworth D, O'Rahilly S, Hivert MF, Loos RJ, Perry JR, et al. A genome-wide association study reveals variants in ARL15 that influence adiponectin levels. PLoS Genet 2009;5:e1000768.
[26] Han LY, Wu QH, Jiao ML, Hao YH, Liang LB, Gao LJ, et al. Associations between single-nucleotide polymorphisms (+45T>G, +276G>T, −11377C>G, −11391G>A) of adiponectin gene and type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetologia)2011;(June) [Epub ahead of print].
[27] Olckers A, Towers GW, van der Merwe A, Schwarz PE, Rheeder P, Schutte AE. Protective effect against type 2 diabetes mellitus identified within the ACDC gene in a black South African diabetic cohort. Metabolism 2007;56:587–92.
[28] Ukkola O, Santaniemi M, Rankinen T, Leon AS, Skinner JS, Wilmore JH, et al. Adiponectin polymorphisms, adiposity and insulin metabolism: HERITAGE family study and Oulu diabetic study. Ann Med 2005;37:141–50.
[29] Wassel CL, Pankow JS, Jacobs Jr DR, Steffes MW, Li N, Schreiner PJ. Variants in the adiponectin gene and serum adiponectin: the Coronary Artery Development in Young Adults (CARDIA) Study. Obesity (Silver Spring) 2010;18:2333–8.
[30] Kaklamani V, Yi N, Zhang K, Sadim M, Offit K, Oddoux C, et al. Polymorphisms of ADIPOQ and ADIPOR1 and prostate cancer risk. Metabolism 2011;60:1234–43.
[31] Hegener HH, Lee IM, Cook NR, Ridker PM, Zee RY. Association of adiponectin gene variations with risk of incident myocardial infarction and ischemic stroke: a nested case-control study. Clin Chem 2006;52:2021–7.
[32] Gu HF, Abulaiti A, Ostenson CG, Humphreys K, Wahlestedt C, Brookes AJ, et al. Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish Caucasians. Diabetes 2004;53(Suppl 1):S31–5.
[33] Wang Y, Zhang D, Liu Y, Yang Y, Zhao T, Xu J, et al. Association study of the single nucleotide polymorphisms in adiponectin-associated genes with type 2 diabetes in Han Chinese. J Genet Genomics 2009;36:417–23.
[34] Chiodini BD, Specchia C, Gori F, Barlera S, D'Orazio A, Pietri S, et al. Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an

association study in an Italian population. Ther Adv Cardiovasc Dis 2010;4:223–30.
[35] Yang M, Qiu CC, Chen W, Xu LL, Yu M, Xiang HD. Identification of a regulatory single nucleotide polymorphism in the adiponectin (APM1) gene associated with type 2 diabetes in Han nationality. Biomed Environ Sci 2008;21:454–9.
[36] Wassel CL, Pankow JS, Rasmussen-Torvik LJ, Li N, Taylor KD, Guo X, et al. Associations of SNPs in ADIPOQ and subclinical cardiovascular disease in the multi-ethnic study of atherosclerosis (MESA). Obesity (Silver Spring) 2011;19:840–7.
[37] Du W, Li Q, Lu Y, Yu X, Ye X, Gao Y, et al. Genetic variants in ADIPOQ gene and the risk of type 2 diabetes: a case-control study of Chinese Han population. Endocrine)2011;(May) [Epub ahead of print].
[38] Ying SY, Lin SL. Intron-derived microRNAs – fine tuning of gene functions. Gene 2004;342:25–8.