ORIGINAL RESEARCH

Influence of socioeconomic lifestyle factors and genetic polymorphism on type 2 diabetes occurrences among Tunisian Arab and Berber groups of Djerba Island

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Abstract: Type 2 diabetes mellitus (T2DM) is characterized by three major metabolic abnormalities: impaired insulin-stimulated glucose uptake in muscle and adipose tissues, alterations in glucose-stimulated insulin secretion, and increased hepatic glucose production. Both genetic and environmental factors contribute to its development. The insulin gene (*INS*), insulin receptor gene (*INSR*), and insulin receptor substrate 1 gene (*IRS1*), identified by polymerase chain reaction and digestion with selected restriction enzymes *PstI*, *NsiI,* and *BstnI,* have been proposed as T2DM candidate genes. To determine the contribution of genetic and environmental factors on the occurrence of T2DM, we examined the frequency of T2DM among two ethnically diverse populations, Arabs and Berbers, who have shared the same environment, the island of Djerba, for thousands of years. Both populations have a high prevalence of obesity, T2DM, and a high consanguinity rate. A total of 162 T2DM men and women were matched to 110 healthy male and female controls. Results showed that the *NsiI* polymorphism in *INSR* and *BstnI* polymorphism of *IRS1* were significantly associated with T2DM only among the Berber group. The *PstI* polymorphism in *INS*, was not associated with T2DM in either group. Sedentary lifestyles, lower physical activity, and lower educational levels were associated with T2DM among the Berber group. These findings suggest that the insulin receptor gene and lifestyle factors in combination may contribute to the occurrence of T2DM in the Berber portion of this island population.

Keywords: polymorphism, insulin, insulin receptor, lifestyle, insulin receptor substrate-1, Berbers, Arabs, Djerba Island

Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disease resulting from an interaction between genetic and environmental factors.[1] Age, physical activity patterns, dietary habits, lifestyle and obesity play a crucial role in the modulation of the disease occurrence. The predisposition to this disease is thought to be conferred by a number of different genes that in isolation may have only minor effects, but in combination lead to the characteristic pathophysiological effects.[2] This genetic susceptibility may be conferred by an unfavorable combination of individual polymorphisms in the genes involved, each one controlling part of the pathogenic process.[3] These genes are defined as "diabetogenes"[4–8] many of which encode proteins of a functional complex that play a key role in glucose metabolism and others involved in the signal transduction characterizing insulin's biological activity.[8] These genes are the insulin

gene (*INS*), the insulin receptor gene (*INSR*), and the insulin receptor substrate 1 gene (*IRS1*)[5] in which several polymorphisms (single-nucleotide polymorphisms [SNPs]) have been described. Some of these SNPs have been associated with T2DM. A polymorphism of interest in the *INS* gene is the *Pst*I RFLP at position 1367 of the 3′ untranslated region.[9,10] In the *INSR* gene, the *Nsi*I RFLP at exon 8, has been associated with arterial hypertension.[11] Finally, the *IRS1* gene presents a polymorphism located at codon 972 (*Bst*n I RFLP),[12] which has been associated with both T2DM and insulin resistance.[12,13]

Studies have investigated polymorphisms associated with T2DM and found that among heterogeneous populations throughout the world, there are similar polymorphisms that convey genetic risks for T2DM in subgroups of main populations, ie, Mexican Americans, Pima Indians, gypsies of southern Slovakia, African Americans, to name a few. This feature has been reported in cases of isolated ethnic groups[14–18] and among disadvantaged groups in developed countries.[19]

Here we focus on well defined neighboring populations belonging to two ethnic groups from the Djerba Island in Southeastern Tunisia. Despite the integration of the socio-culturally diverse civilizations over hundreds of years, ethnic separation remains a part of everyday life in Tunisia, mostly due to the different practices of the Muslim religion. Thus, the two populations maintain their separate cultures and ways of life although they share an agricultural and artisans economy. Despite their differences, both Arab and Berber groups show a high prevalence of T2DM, obesity, high consanguinity rate, and metabolic syndrome characteristics. Consequently, this environment provides the opportunity to investigate the effect of lifestyle on T2DM occurrence in association with genetic polymorphisms. We, therefore, randomly sampled the population to examine RFLPs at the *INS*, *INSR*, and *IRS1* genes and the correlation between T2DM and particular environment risk factors for each ethnic group.

Materials and methods
Subjects
Unrelated men and women of Arab and Berber descent (172 Arab Tunisians and 100 Berber Tunisians) originating from the Island of Djerba were recruited from the Department of Endocrinology of Midoun and Houmet Essouk Hospital (for Arabs) and the Department of Endocrinology of Guellala and Sedwich Hospital (for Berbers). Healthy controls were recruited from the local villages throughout the Island.

Arabs and Berbers were divided into two groups according to their diagnosis of T2DM. Inclusion criteria required that participants were of adult age, had lived on the island their entire lives, and that their partner was of the same ethnicity and had been born and raised on the island as well.

Clinical intake and medical histories
Clinical features and family history of diabetes were collected by questionnaire and interviews. Fasting glycemic status was checked for each participant. Randomly selected individuals had fasting glucose <6.1 mmol/l and did not suffer from any other disease. Weight and height were measured on barefoot and lightly clothed subjects. Body mass index (BMI; kg/m^2) was calculated and obesity was defined as BMI $\geq$ 30 kg/m^2.[19] Hypertension was defined as a systolic pressure $\geq$140 mmHg or diastolic pressure $\geq$90 mmHg. The characteristics of the two groups are presented in Table 1. Venous blood samples were drawn for determination of plasma glucose and DNA extraction.

Questionnaires and interviews
For each subject, a questionnaire about lifestyle, age, education level, and physical activity was administered. Usual dietary intake was assessed with the use of a semi-quantitative food-frequency questionnaire (FFQ). The FFQ consisted of a list of foods with a standard serving size. Participants were asked to report their frequency of consumption of each food item during the previous year on a daily, weekly or monthly basis. Portion sizes of consumed foods were converted from household measures to grams. Each food and beverage was then coded according to the prescribed protocol and analyzed for content of energy and the other nutrients by weight using Bilnut software (INSERM; Nutrisoft, Cerelles, France), which was adapted for Tunisian foods.

Two measures of socioeconomic level status were used: 1) Level of education was measured as number of years spent in the education system, eg, primary school, secondary school, and high school, in contrast to nonreading analphabets. 2) Socioeconomic status was based on a questionnaire that asked about material possessions within the household, eg, water pipeline; two TV sets, toilet that can be flushed, bathroom, computer, gas/central heating, telephone, car, freezer, another apartment, video recorder.

For physical activity assessment, subjects completed a physical-activity-recall questionnaire. They were asked to record whether they are sleeping, sitting, standing, or watching television during each hour of the day. Subjects noted the time of day they started each new activity, body position

Table 1 Sociodemographic and lifestyle characteristics of the different ethnic groups

| | Berber group | | | | | | Arab group | | | | | |
| | Men | | | Women | | | Men | | | Women | | |
	T2DM	Controls	p	T2DM	Controls	p	T2DM	Controls	p	T2DM	Controls	p
Total	N = 26	N = 12		N = 34	N = 28		N = 44	N = 31		N = 58	N = 39	
Age (years)	63.5 ± 11.1	55.42 ± 14.0	NS	55.9 ± 11.5	51.5 ± 13.3	NS	58.02 ± 10.7	54.2 ± 12.8	NS	57.9 ± 9.7	54.1 ± 12.5	NS
Low education level	34.62%	16.67%	<0.05	67.65 %	71.43 %	NS	34.1%	51.6%	NS	60.34%	48.72%	NS
Sedentary lifestyle	46.15%	50%	NS	2.94%	0 %	<0.05	65.91%	77.42%	NS	13.79%	10.26%	NS
BMI (Kg/m²)	26.71 ± 3.75	26.29 ± 5.86	NS	30.19 ± 5.69	30.46 ± 4.23	NS	26.70 ± 4.40	27.66 ± 4.28	NS	27.37 ± 5.68	27.98 ± 5.40	NS
Height (cm)	162 ± 8	163 ± 9	NS	155 ± 9	153 ± 7	NS	168 ± 7	167 ± 8	NS	161 ± 7	161 ± 9	NS
Weight (kg)	69.7 ± 8.7	68.9 ± 16.2	NS	72.0 ± 13.8	73.7 ± 13.5	NS	75.3 ± 11.6	79.0 ± 13.8	NS	71.3 ± 13.5	72.7 ± 13.0	NS
Low socioeconomic level	65.38%	33.33%	<0.05	100%	96.43%	<0.05	59.09%	51.61%	NS	94.83%	84.62%	NS
Low physical activity	34.61%	0%	<0.05	20.59%	7.14%	<0.05	20.45%	9.67%	NS	24.13%	12.82%	NS
Smoking habits	11.53%	25.00%	NS	5.88%	0	NS	31.81%	32.25%	NS	6.89%	2.56%	NS
Alcohol consumption	11.53%	33.33%	NS	0	0	NS	13.63%	9.67%	NS	0%	0%	NS
HTA	61.54%	8.33%	<0.05	58.82%	14.29%	<0.05	52.27%	9.68%	<0.05	39.66%	25.64%	NS
PD	8.73 ± 2.05	8.00 ± 0.60	NS	8.11 ± 0.76	8.17 ± 0.77	NS	8.02 ± 0.92	7.80 ± 0.70	NS	8.11 ± 0.61	8.07 ± 0.83	NS
PS	14.50 ± 1.90	12.75 ± 1.35	NS	14.14 ± 5.34	13.32 ± 1.92	NS	13.84 ± 1.39	12.87 ± 0.99	NS	13.75 ± 1.97	12.94 ± 1.23	NS
G0	1.62 ± 0.39	0.93 ± 0.08	NS	1.59 ± 0.15	0.93 ± 0.08	NS	1.71 ± 0.39	0.95 ± 0.08	NS	1.71 ± 0.48	0.93 ± 0.11	NS
GPP	2.61 ± 0.27	1.83 ± 0.22	NS	2.75 ± 0.87	1.79 ± 0.28	NS	2.53 ± 0.73	1.87 ± 0.28	NS	2.73 ± 0.29	1.77 ± 0.26	NS

Notes: Data are mean ± standard deviation or percentage frequency, age and sex are adjusted only for ethnicity; p < 0.05.
Abbreviations: BMI, body mass index; GO, fasting glycemia; GPP, 2-h glycemia; HTA, hypertension; NS, not significant; PD, diastolic pressure; PS, systolic pressure; T2DM, type 2 diabetes mellitus.

during the activity (reclining, sitting, standing, or walking), effort (light, moderate, vigorous). They were asked about both leisure and occupational physical activity performed over the past week. For the past week, the average number of hours per week of each activity was calculated. The total hours of each activity were multiplied by the estimated metabolic cost (EMC) of the activity then, the physical activity level (PAL) was computed as the total energy expended over 24 hours divided by 24. Gender, age, height and body weight are the main determinants of basal metabolic rate (BMR). To estimate the BMR we used predictive equations based on these factors. By multiplying the PAL by the BMR, the total energy expenditure (TEE) was determined.

All procedures involved in the current study were reviewed and approved by the Ethics Committee of the Medicine University of Tunis. All study subjects signed informed consents.

Methods

Genomic DNA was isolated from peripheral blood by phenol extraction.[20] Polymerase chain reaction (PCR) was performed using three primer pairs corresponding to respectively: the 3′ untranslated region of the *INS* gene, exon 8 of the *INSR* gene, and the region encompassing codon 972 of the *IRS1* gene. Two sequence-specific oligonucleotide primers were used for the polymerase chain reaction: the 3′ primer (5′AGGCGTGGCATTGTGCCA3′) was used in combination with the 5′ primer (5′CTGGGAGGGGCTC-CAAGT 3′) for the insulin gene, for the receptor of the insulin we used the 3′ primer (5′CGGTCTTGTAAGGG-TAACTG3′) was used in combination with the 5′ primer (5'GAATTCACATTCCCAAGACA3') and for the insulin receptor substrate 1 gene we used the 3′ primer (5′CTTCT-GTCAGGTGTCCATCC3′) was used in combination with the 5′ primer (5′TGGCGAGGTGTCCACGTAGC3′) These primers have been designed at Centro de Investigación Biomédica de Occidente in Mexico. Amplified products were digested with the corresponding restriction enzymes: *Pst*I for the *INS* gene, *Nsi*I for the *INSR* gene, and *Bstn*I for the *IRS1* gene amplicons. The digestion products were resolved by polyacrylamide gel electrophoresis and visualized by silver staining.[21,22]

Statistical analysis

The statistical software SPSS version 10.0 (SPSS Inc., Chicago, IL, USA) was used to analyze variables of the Arab and Berber groups relative to T2DM. The EPI INFO 6 package program (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used to compare allelic and genotypic frequencies in diabetic patients and controls performing the Student's *t*-test, the Pearson's χ^2 tests and the Fisher's exact test (when genotype class was below 5). Odds ratios (OR) are given with their corresponding 95% confidence intervals (CI). A p value of <0.05 was considered to be statistically significant. Intakes were calculated with Bilnut software (Bilnut 4.0; Nutrisoft), which was adapted for Tunisian foods, for total energy, protein, carbohydrates (total, sucrose), fat (total, saturated, poly and monounsaturated), fiber, calcium, magnesium, sodium, phosphorus, animal to vegetable protein ratios, and polyunsaturated to saturated fat ratios.

Results

Sociodemographic and lifestyle characteristics

We compared the clinical (BMI and arterial pressure) characteristics of 162 T2DM patients and 110 healthy control subjects belonging to Berber and Arab groups of Djerba Island (Table 1). The mean age of the Arab T2DM group was 57.96 ± 10.09 and 54.13 ± 12.53 years for Arab controls. The mean age of the Berber T2DM group was 59.2 ± 11.85 and 52.70 ± 13.44 years for controls. Age was not significantly different for the groups as expected due to the matching design used. Results revealed a significant difference in educational levels between these two groups. In comparison with the Arabs, the Berbers had a lower level of education (Table 1) with a significant difference between Berber men and women. The Berber T2DM male's socioeconomic level was significantly higher (p < 0.050) than controls. There was no significant difference in the Arab groups.

The prevalence of low physical activity was significantly higher among the Berber male group than the Arab male group; likewise, the prevalence of sedentary lifestyle was higher among the Berbers group compared to that of the Arabs. There was no difference in alcohol consumption and no significant difference in smoking habits between patients and controls (Table 1).

Caloric intakes

Table 2 presents mean dietary intake by ethnic groups for males and females, respectively. Mean caloric intakes among Arab males were 1846 ± 262 kcal. Caloric intakes among Berber males were 2039 ± 316 Kcal. For females, caloric intakes were 1833 ± 254 Kcal for Arabs and 1858 ± 239 kcal for Berbers. Mean caloric intakes for Arab subjects were

1839 ± 256 while it was 1927 ± 283 for Berber subjects. There were no significant ethnic differences in caloric intake in either sex group.

Macronutrient intakes

Average protein intake for each group was 56 g, 65 g, 55 g, and 57 g for Arab men, Berber men, Arab women, and Berber women, respectively (Table 2). There was no significant difference among women of either groups when compared by ethnicity but there was a significant difference (p < 0.02) between Arab and Berber men. Nevertheless, the animal protein to vegetable protein ratio was significantly greater in the Arab group (men and women) than Berber group (p < 0.03).

Average carbohydrate consumption showed no statistical significance across groups when compared by ethnicity. However the Arab group consumed more sucrose than the Berber group (p < 0.02) and the Berber group consumed more fiber than the Arab group (p < 0.02). There was a positive correlation between calories and fiber intake (r = 0.55; p < 0.001) and a negative correlation between fiber and sucrose intake (r = −0.33; p < 0.001).

Prevalence of overweight and of reported morbidity

Prevalence of overweight was higher among the Berber women than among the Arabs, but the difference was not significant between T2DM cases and controls (Table 1). There was no significant difference in height and weight between the two groups. There was a significant difference in hypertension in the two groups. There was no significant difference between diastolic and systolic pressure.

Comparison between Arab and Berber allelic and genotypic distribution

Polymorphism of three genes was analyzed in 162 patients and 110 controls: *PstI* polymorphism in nucleotide 1367 of the 3′ untranslated regions of the insulin gene,[23] *NsiI* polymorphism in exon 8 at nucleotide 6224 of the insulin receptor gene[24] and *BstnI* polymorphism at codon 972 in the coding region of the insulin receptor substrate 1 gene.[25]

To assess associations between these described polymorphisms and T2DM, we analyzed the allelic and genotypic frequencies in patients and healthy controls belonging to Arab and Berber subgroups in Djerba.

Table 2 Dietary intakes in different ethnic groups

	Arab group			Berber group		
	Men	**Women**	**Total**	**Men**	**Women**	**Total**
Energy expenditure (cal/day)	2293 ± 230	1825 ± 154	2044 ± 304	2404 ± 574	1832 ± 188	2027 ± 454
Total energy intake (cal/day)	1966 ± 185	1922 ± 215	1943 ± 201	2069 ± 232	1951 ± 152	1991 ± 189
Protein (g/day)	56 ± 10	55 ± 11	55 ± 11	65 ± 14[3]	57 ± 11	60 ± 13
Protein (% TEI)	12.1 ± 1.5	12.1 ± 1.8	12.1 ± 1.6	12.8 ± 1.7	12.3 ± 1.5	12.5 ± 1.5
AP/VP	0.81 ± 0.30	0.80 ± 0.33	0.81 ± 0.32[1]	0.71 ± 0.44	0.65 ± 0.27	0.67 ± 0.30
Fat (g/day)	68 ± 18	70 ± 14	69 ± 16[1]	83 ± 19[3]	70 ± 14	78 ± 17
Fat (% TEI)	33.5 ± 6.0	34.5 ± 3.8	34.0 ± 4.9[1]	36.7 ± 4.7	36.4 ± 4.4	36.5 ± 4.5
SFA (g/day)	17.4 ± 5.8	18.2 ± 4.9	17.7 ± 5.3	18.8 ± 4.8	17.9 ± 5.6	18.2 ± 5.3
SFA (% TEI)	7.8 ± 2.3	8.5 ± 2.0	8.2 ± 2.2	8.2 ± 1.5	8.2 ± 2.3	8.2 ± 2.0
MUFA (g/day)	43.6 ± 12.1	43.3 ± 9.7	43.4 ± 10.7[1]	53.5 ± 13.4[3]	48.4 ± 10.5	50.1 ± 11.7
MUFA (% TEI)	19.8 ± 4.7	20.1 ± 3.1	20.0 ± 3.9[1]	23. ± 3.5[3]	22.2 ± 3.7[2]	22.5 ± 3.6
PUFA (g/day)	7.5 ± 1.9	8.2 ± 2.8	7.9 ± 2.4[1]	10.7 ± 5.6[3]	9.2 ± 3.1	9.7 ± 4.1
PUFA (% TEI)	3.4 ± 0.8	3.8 ± 1.1	3.6 ± 1.0[1]	4.1 ± 1.1	4.2 ± 1.4	4.2 ± 1.3
PUFA/SFA	0.47 ± 0.13	0.46 ± 0.13	0.47 ± 0.13[1]	0.51 ± 0.14	0.55 ± 0.20[2]	0.54 ± 0.18
Cholesterol (mg/day)	127.4 ± 100.1	101.4 ± 76.1	113.6 ± 88.1	94.3 ± 84.1	109.8 ± 98.9	104.6 ± 93.5
Carbohydrates (g/day)	251 ± 43	244 ± 44	248 ± 42	253 ± 35	236 ± 28	242 ± 31
Sucrose (g/day)	34 ± 22	33 ± 22	33 ± 22[1]	18 ± 12[3]	25 ± 23	23 ± 20
Fibres (g/day)	25 ± 5	24 ± 7	24 ± 6	29 ± 7[3]	25 ± 5	27 ± 7

Notes: [1]p < 0.05 between Arab and Berber groups; [2]p < 0.05 between Arab and Berber women; [3]p < 0.05 between Arab and Berber men.
Abbreviations: AP/VP, animal protein/vegetal protein ratio; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; TEI, total energy intake; PUFA/SFA = poly unsaturated fatty acid-saturated fatty acid ratio.

The genotype distributions for these SNPs were found to be in Hardy–Weinberg equilibrium for these populations. The allelic distributions for *PstI*, *NsiI*, and *BstnI* polymorphisms in cases and control groups are summarized in Table 3. The comparison of the allelic distribution between the four subgroups has demonstrated a significant difference ($p < 0.05$) for the *NsiI* polymorphism of the INSR gene and *BstnI* polymorphism of IRS1 gene only for the Berber group. The effect of the *NsiI* G and A alleles ($OR_{INSR\ NsiI\ G} = 0.52$, 95% CI: 0.26–2.01; $p = 0.037$ and $OR_{INSR\ NsiI\ A} = 1.93$, 95% CI: 0.99–3.79; $p = 0.037$, respectively) seems to be protective against T2DM in Berbers only.

Genotype distribution for these polymorphisms in T2DM cases and control groups were summarized in Table 4. It appeared that *INSR NsiI (G/G)* and *IRS1BstnI (G/A)* SNPs could be protective against T2DM ($OR_{INSR\ NsiI\ G/G} = 0.48$, 95% CI: 0.17–2.05; $p = 0.04$ and $OR_{IRS1BstnI\ G/A} = 0.37$, 95% CI: 0.14–2.02; $p = 0.032$, respectively) in Berbers, since these genotypes were more frequent in healthy controls than in Berber T2DMs (67% versus 57%, 5% *for NsiI* [G/G] and 18.33% versus 37.5% for *BstnI* [G/A]). The *PstI* SNP was not associated with T2DM in either of these two subgroups.

Discussion

In the present work, we investigated the effect of lifestyle and socioeconomic factors on T2DM occurrence, in association with genetic polymorphisms. Our choice of Arab and Berber ethnic groups of Djerba Island is based on previous observations indicating that these populations, though living in the same environment and displaying the same genetic background, have different lifestyles in terms of activity, culture, lifestyle, and nutrition. The Berber and Arab communities, which constitute a minority of the Tunisian population, are isolated from the other two population groups who have settled in the villages of the Djerba Island.[26,27] We have focused on the *PstI, NsiI,* and *BstnI,* restrictions sites of the insulin, insulin receptor, and insulin receptor substrate 1 genes, respectively. These genes are known to play a key role in glucose and lipid metabolism.

We examined possible correlations between these polymorphisms and the predisposition to T2DM. We have shown that the healthy Arab and Berber populations display the same allelic and genotypic distributions and that the *Pst1* polymorphism of the *INS* gene is not associated with T2DM in either studied groups. In fact, it has been reported that polymorphisms in a variable number of tandem repeat (VNTR) sequences in *INS,* the insulin gene, cause type 1 diabetes.[28] This region regulates insulin gene transcription thus providing a mechanism whereby this gene may be related to hyperinsulinemia, and thereby to T2DM or metabolic syndrome. Polymorphisms in *INS* are associated with T2DM in some populations,[29] but not in others.[30]

On the other hand, we found an association of *NsiI* insulin receptor gene polymorphism with T2DM in the Berber group. In the literature, mutations of the insulin-receptor gene have been reported in patients with the rare syndrome of severe insulin resistance that contributes to noninsulin-dependent diabetes mellitus.[31] Polymorphisms in this receptor gene are associated in some populations with T2DM, insulin resistance,[32] and hypertension.

Finally we have shown that the *BstnI* Gly 972 Arg polymorphism of the insulin receptor substrate 1 is associated

Table 3 Allelic distribution of *Psti*, *Nsi*, and *Bstni* polymorphisms in Arabs and Berbers of Djerba Island

Gene symbol	Alleles	Djerbian diabetic patients N = 162		Djerbian controls N = 110		Arab patients vs Arab controls p value, OR (confidence Interval)	Berber patients vs Berber controls p value, OR (confidence Interval)
		Arabs N = 102%	Berbers N = 60%	Arabs N = 70%	Berbers N = 40%		
	PstI						
INS	G	93.63	92.5	91.43	87.5	NS	NS
	A	6.37	7.5	8.57	12.5	NS	NS
	NsiI						
INSR	G	72.55	60.83	74.28	75	NS	p = 0.037; OR = 0.52 (0.26–2.01)
	A	27.45	39.17	25.72	25	NS	p = 0.037; OR = 1.93 (0.99–3.79)
	BstnI						
IRS1	G	83.82	89.17	84.28	81.25	NS	NS
	A	16.18	10.83	17.72	18.75	NS	NS

Notes: Nominal value for comparison, $p \leq 0.05$; degree of freedom = 1.
Abbreviations: NS, not significant; OR, odds ratio.

Table 4 Genotypic distribution of, *PstI*, *NsiI*, and *BstnI* polymorphisms in Arabs and Berbers of Djerba Island

Gene symbol	Genotypes	Diabetic patients N = 162		Controls N = 110		Arab diabetics vs Arab controls p value; OR (confidence interval)	Berber diabetics vs Berber controls p value; OR (confidence interval)
		Arabs N = 102%	Berbers N = 60%	Arabs n = 70%	Berbers n = 40%		
	PstI						
INS	G/G	87.25	85	82.86	75	NS	NS
	G/A	12.75	15	17.14	25	NS	NS
	A/A	0	0	0	0	NS	NS
	NsiI						p = 0.04; OR = 0.43;
INSR	G/G	51.96	36.67	54.28	57.5	NS	(0.17–2.05)
	G/A	41.17	48.33	40	35	NS	NS
	A/A	6.87	15	5.72	7.5	NS	NS
	BstnI						
IRS1	G/G	67.65	80	68.57	62.5	NS	NS
	G/A	32.35	18.33	31.43	37.5	NS	p = 0.032; OR = 0.37;
	A/A	0	1.67	0	0	NS	(0.14–2.02)
							NS

Notes: Nominal value for comparison, p ≤ 0.05; degree of freedom = 1.
Abbreviation: NS, not significant.

with T2DM only among Berbers. As reported in the literature, mutations of the insulin-receptor substrate gene have been described in patients with the rare syndrome of severe insulin resistance that contributes to noninsulin-dependent diabetes mellitus.[33,34] Indeed several studies have reported a higher frequency of the *IRS1* Arg 972 polymorphism among T2DM patients, while others have reported a weak association or its absence between this variant and T2DM.[35] The *IRS1* Arg 972 polymorphism is absent in Pima Indians and other subpopulations.[36–38] In both diabetic and control Japanese populations, the prevalence of the Arg 972 polymorphism appears to be lower than that observed in Caucasian populations.[39] These results may account for the ethnic differences in the genetic background for T2DM. There were no differences between males and females in their respective clinical and biochemical findings, although there was a slightly higher prevalence of obesity in Berber women (based on BMI, height, and weight data), also characterized by a low education level and a low activity or sedentary lifestyle. A significant difference (p < 0.05) in education level, sedentary lifestyle, low physical activity, and socioeconomic levels between healthy and diabetic Berber male subjects was found, suggesting that correlations may exist among these factors and risk for T2DM among Berbers. For the Arab group, no significant difference was observed between healthy and T2DM subjects. Hypertension was associated with T2DM in both groups.

To explain the differences between our results on the two Djerban groups and to distinguish genetic factors from the influence of the environment factors, we considered the nongenetic factors, ie, factors that can be controlled by behavior, (diet, activity levels/sedentary lifestyle, and obesity) implicated in the etiology of diabetes. These environmental factors are significantly different between Arabs and Berbers. The presence of some risk factors for T2DM in diabetic Berbers are related to lifestyle such as a more sedentary lifestyle, lower levels of physical activity, and lower levels of education for diabetic males who do not complete primary education and who are of a lower socioeconomic status. Berbers who have an underprivileged social background have higher rates of mortality and morbidity compared to the Arabs; they retain traditional behaviors that result in T2DM.[40] Social status was found to be a risk factor for T2DM in other populations such as those in urban Sweden.[41] In fact, our samples were obtained from public hospitals in villages where most patients are from a lower socioeconomic background.

We have shown that in the Berber group, low levels of education, socioeconomic status and limited physical activity are risk factors for T2DM. *INSR* gene *NsiI* G/G and *IRS1* gene *BstnI G/A* genotype are protective against T2DM. For the Arab group, these same factors are not significantly associated to T2DM. More investigation is needed to analyse the occurrence of T2DM in Arabs to determine the source of their T2DM protection, or other environmental and genetic risk factors. To our knowledge this is the first study which compares the dietary intake of Arab and Berber people. The inclusion criteria of this study reduced the influence of the socioeconomic level and

acculturation on the food choices. These conditions were often considered as factors of variability. Low education level is a factor that exacerbates the problem of obesity in Tunisia. Women without education do not recognize the risks and health consequences associated with obesity. It is more worrisome that these women (and their male partners) consider fatness and obesity to be desirable since culturally these traits have been associated with higher social status, fertility and prosperity.

In conclusion, these results focus on some of the risk factors leading to T2DM and on the possible interactions between genetic and environmental factors. Ethnicity and differences in education level play an important role in the occurrence of the disease.[42] Berbers may constitute a group at higher risk for developing T2DM and hypertension, due to their nutritional habits. Lack of adequate dietary intake, accompanied by obesity, low physical activity and low socioeconomic levels may combine to cause this disease. These findings suggest that appropriate dietary interventions and education may ameliorate nutritional deficiencies and improve healthy behavioral lifestyles.

Acknowledgments

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