Contribution of Type 2 diabetes associated loci in the Arabic Population from Tunisia and trans-ethnic genetic heterogeneity

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ABSTRACT

Background. Candidate gene and recent genome-wide association studies have both reproducibly identified several common Single Nucleotide Polymorphisms (SNPs) that confer type 2 diabetes (T2D) risk in European populations. Our aim was to evaluate the contribution to T2D of five of these established T2D-associated loci in the Arabic population from Tunisia.

Methods. A case-control design comprising 884 type 2 diabetic patients and 513 control subjects living in the East-Center of Tunisia was used to analyze the contribution to T2D of the following SNPs: E23K in KCNJ11/Kir6.2, K121Q in ENPP1, the -30G/A variant in the pancreatic β-cell specific promoter of Glucokinase, rs7903146 in TCF7L2 encoding transcription factor 7-like2, and rs7923837 in HHEX encoding the homeobox, hematopoietically expressed transcription factor. Their effects on quantitative metabolic traits in the normoglycemic subjects were also assessed.

Results. TCF7L2-rs7903146 T allele increased susceptibility to T2D (OR = 1.25 [1.06-1.47], P = 0.006) in our study population. This risk was 56% higher among subjects carrying the TT genotype in comparison to those carrying the CC genotype (OR = 1.56 [1.13-2.16], P = 0.002). No allelic or genotypic association with T2D was detected for the other studied polymorphisms.

Conclusions. In the Tunisian population, TCF7L2-rs7903146 T allele confers an increased risk in T2D as previously reported in the Caucasian population and many other ethnic groups. In contrast, none of the other tested SNPs that influence T2D risk in the European population was associated with T2D in the Tunisian Arabic population. An insufficient power to detect minor allelic contributions or genetic heterogeneity of T2D between different ethnic groups can explain these findings.
Introduction

T2D is a complex metabolic disorder which is caused by both decreased insulin sensitivity, and impaired insulin secretion due to pancreatic β-cell defects [1]. T2D is thought to result from the effects of environmental and lifestyle risk factors together with at-risk genetic variants in predisposed individuals [2].

T2D is a global major health problem showing worldwide increasing prevalence [3]. The Arabic population is however particularly targeted by T2D [4, 5]. In the Tunisian population, the prevalence of T2D reaches 9% of adults [6] that is much higher compared with European populations and may be due to the specificity of the Tunisian life style or to a specific genetic background.

From the previous familial linkage and candidate-gene studies, T2D-associated single nucleotide polymorphisms (SNPs) have been confirmed and widely replicated, but with modest effects on disease risk [7, 8]. These variants include the E23K variation in KCNJ11, encoding the Kir6.2 subunit of the K⁺-ATP channel [9], the Pro12Ala variant in PPARG [10], the -30G/A polymorphism in the β-cell specific promoter of glucokinase (GCK) [8, 11], and the K121Q variant of ENPP1 encoding ectonucleotide pyrophosphatase phosphodiesterase, the inhibitor of insulin receptor [12]. The SNP with the largest risk effect is the intronic variant, rs7903146, in the TCF7L2[SC1] locus [13, 14]. This association was consistently replicated in populations of various ethnic origins, among which Morroccans [15].

Recently, genome-wide association (GWA) studies revealed novel SNPs that increased T2D risk in different European populations [14, 16, 17, 18]. The French GWA study detected unexpected association to T2D for non-coding SNPs at the HHEX locus (homeobox, hematopoietically expressed) [14], which were also shown to contribute to an increased risk of T2D in British [18], Japanese [19] and other Asiatic [20] populations.
In this study, we analyzed five polymorphisms in the following genes, rs7903146 of TCF7L2, rs7923837 of HHEX, rs1788994 of GCK, rs5219 of KCNJ11/Kir6.2 and rs1044498 of ENPP1 using a case-control design in 1,397 individuals (884 unrelated T2D patients and 513 normoglycemic controls) in order to evaluate their impact on T2D risk in the Tunisian population living in the East-Center part of the country.

**Methods**

**Study population**

The T2D group includes 884 unrelated Tunisian diabetic subjects (406 males, 478 females). The affected individuals were recruited in 2003-2006 in collaboration with the Endocrinology-Diabetology departments of Farhat Hached Hospital (Sousse, Tunisia) and Fattouma Bourguiba University Hospital (Monastir, Tunisia). T2D was defined according to 1997 American Diabetes Association. Inclusion criteria: fasting plasma glucose $\geq 7.0$ mmol/l and/or treatment for diabetes included diet and/or oral antidiabetic drugs and/or insulin to achieve glycemic control. All subjects who required insulin had been treated with oral drugs for at least 2 years.

Individual and clinical characteristics were recorded for all subjects, including age at examination, gender, age at diagnosis, duration of diabetes, first-degree family history of diabetes, treatment for diabetes including date of initiation and/or discontinuation of oral agents or insulin. When available, the following details were obtained from the clinic records: dyslipidaemia, history of chronic complications of diabetes, history of hypertension, ischaemic heart disease and other medical illness.

All T2D patients were compared to a group of 513 normoglycaemic subjects (fasting glycaemia < 6.1 mmol/l, age at examination > 45 years, BMI < 30 kg/m$^2$) from blood donors recruited in the transfusion centres of Monastir and Sousse (Center of Tunisia). None was first degree relative of
other subjects in the case or control groups; they were not known to have diabetes although occult disease was not excluded.

Written informed consent was obtained from all subjects and DNA was extracted using the standard phenol-chloroform procedure. The study protocol was approved by the University of Monastir (Tunisia).

**SNP genotyping**

SNP genotyping of rs7903146 in *TCF7L2*, rs7923837 in *HHEX* and rs1799884 in *GCK* promoter were performed using allelic discrimination TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, Calif. USA). The PCR primers and TaqMan probes were designed by Primer Express and optimized according to the manufacturer's protocol. We obtained a 95% genotyping success rate (except for *HHEX* rs7923837 which gave a 90% genotyping rate). A random of 10% sample set was re-tested with the same method to confirm genotype accuracy. No difference of genotypes was found between the duplicate samples.

For genotyping of E23K (rs5219) in *KCNJ11/Kir6.2* and K121Q (rs1044498) in *ENPP1*, we used the FRET technology using the Light Cycler TM assay (Roche Diagnostics, Basel, Switzerland). For both SNPs, the genotyping success rate was 91%. In order to assess genotyping accuracy for these two SNPs, 20 random samples were tested by direct sequencing, which provided a 100% concordance rate.

**Statistical analysis**

Allele frequencies were calculated by the genotype-counting method, and each polymorphism was tested for Hardy-Weinberg equilibrium using Chi square goodness-of-fit test using HPlus 2.5 software. Comparison of allele frequencies and genotype distributions between all T2D and control groups were done using the Pearson's Chi square test.
Genotypic associations for additive, dominant and recessive models were tested by calculating a logistic regression (adjustments) statistic and corresponding $P$ value using the program SNPstats [http://bioinfo.iconcologia.net/index.php?module=Snpstats]. The results are expressed as $P$ value (two-tailed), odds ratio (OR) and 95% confidence intervals (CI). The minimum detectable effect size with a statistical power of 80% was assessed [21] using Quanto software v.1.2.3 [http://hydra.usc.edu/GxE].

Student’s t-test, used to determine differences in means of continuous variables in the normoglycemic control subjects, was performed using the SPSS statistical analysis software v.16.0 (SPSS, Chicago, IL). Statistical significance was set at a $P$-value $< 0.05$.

**Results**

The clinical characteristics of the T2D patients and control subjects are given in Table 1. Among T2D subjects, 30.09% ($n = 266$) are obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) versus 69.91% ($n = 618$) non-obese ($\text{BMI} < 30 \text{ kg/m}^2$). No significant differences were noted when all T2D patients were compared to the non-obese T2D group for clinical features (data not shown).

The distribution of allelic and genotypic frequencies of the five SNPs was compared between the two study groups (884 T2D cases and 513 normoglycemic controls) (Table 2). The genotype distributions of all SNPs obeyed Hardy Weinberg equilibrium in the control group (Supplementary Table 1).

The T allele of *TCF7L2* was significantly associated with increased risk of T2D and the OR adjusted for age, gender and BMI was 1.25, 95% IC [1.06-1.47], $P = 0.006$ (Table 2). The association was observed in all genotypic models but the highest risk was observed under the allelic model (OR = 1.56, 95% IC = 1.13-2.16, $P = 0.002$) (Table 2).

As shown in Table 2, no other alleles of the remaining variants were associated with an increased risk of T2D in the Tunisian population.
An estimation of the minimum effect size detected with a statistical power of 80% under different genetic models, and according to the allelic frequencies of each SNP tested in our study, is presented in the Supplementary Table 2. Except for TCF7L2 SNP that showed a significant effect on T2D risk with an additive OR value above the threshold estimated in our power calculation, the other four SNPs are known to have a lower allelic contribution that could not be easily detectable in this middle-sized cohort.

In order to evaluate whether the genetic variants studied above interacted with quantitative metabolic traits, we also analyzed by linear regression the correlation between genotypes at the five SNPs and quantitative metabolic traits (like fasting glucose level, HbA1C) in the normoglycemic control group. None of the SNPs tested were associated with these metabolic variables under all genotypic models (data not shown).

Discussion

The T-allele of TCF7L2-rs7903146 is associated with an increased risk in T2D among the Tunisian population. Recent GWA studies in populations of European descent showed that TCF7L2 is the T2D gene having the largest risk effect to date [14, 22], even if the causative variant(s) and etiological mechanism(s) are not yet completely characterized [23]. Our data are also in agreement with the numerous previous replications of the TCF7L2-associated SNPs mostly in white Europeans, but also in West Africans, Mexican and African Americans, Indians and Japanese populations [13, 15, 24]. The rs7903146 variant was also associated with an increased risk in T2D in Moroccan subjects, suggesting a similar effect in several North African populations [15]. However, a recent study of TCF7L2 variants in an Arab population of Saudi origin reported no association with T2D upon analysis of two SNPs (including rs7903146) [25].
With regard to the remaining four loci, no association with T2D was detected for \textit{HHEX}, \textit{GCK}, \textit{KCNJ11} and \textit{ENPP1} variants, whereas previous independent studies reported such associations with T2D risk in several European white populations [9, 14, 26, 27]. However, a lack of association with T2D was also reported for the \textit{HHEX} variants in the Moroccan cohort [27].

The extensively studied K121Q variant (rs1044498) in \textit{ENPP1} did not reveal evidence for association with T2D in our study from the Tunisian population. The contribution of this variant to T2D risk in the European white populations has been well established in several [12, 28] but not all [29, 30] studies. These findings question the reproducibility of a real contribution to T2D risk and emphasize the more complex diabesity component.

A recent study reported that the \textit{ENPP1}-K121Q variant may predispose to T2D in their Tunisian study population (with an OR of 1.89, 95\%CI [1.13–3.15], under a dominant model) [31], but the smaller sample size and ascertainment of the diabetic patients analyzed in this study could explain the observed difference. In addition, it is noteworthy that in our study we investigated a multicentric sample of T2D patients, more representative of the Tunisian general population contrary to the Bouhaha study group’s which is only from the north of Tunisia. In this part of country, people lifestyle’s is more westernized with reduced physical activity and excessive calorie intake in foods. Otherwise, these divergent results of association, whereas a similar frequency of the Q-121 allele was observed in both studies, could be explained by a modest contribution of the \textit{ENPP1}-K121Q variant to the risk of T2D in Tunisians at the population level, and also by interactions with BMI or environmental factors in modulating the risk of T2D, as previously reported [32].

We have to note that the present study was underpowered to demonstrate similar effects in T2D risk than those previously reported for \textit{HHEX}, \textit{GCK}, \textit{KCNJ11} or \textit{ENPP1} variants by several meta-analyses and independent studies from the European diabetic cohorts [27,28,33]. Indeed, more modest genetic effects in a polygenic context will need to analyze a larger sample size to be able to definitely conclude between a lack of association and genetic heterogeneity due to a specific ethnic
background and an insufficient power in the association study. Otherwise, the modulation by the overweight or obesity status of the genetic effects of variants associated either with insulin secretion (like for *GCK, KCNJ11, or TCF7L2*) or insulin action (for *ENPP1, ADIPOQ or PPARG*) in T2D risk was previously well established [9,12,26,29,30,33], with the demonstration that the T2D risk effect is different in obese and non-obese individuals depending on the at-risk variant [12,26,30,33]. Thus, it is also important to consider such interaction effects in the study design and sample size when analyzing gene variants with minor genetic effects on T2D risk.

In conclusion, our data support an effect of the widely replicated TCF7L2 variant on T2D risk in the Arabic population from Tunisia, whereas the other variants tested were not found to play a major role in T2D. These different findings from the European and non-European populations can be explained by several factors, such as a minor contribution that is not detectable in this middle-sized cohort, the presence of Arabic-specific SNPs in some loci, or a genetic heterogeneity of T2D between different ethnic groups, which we already highlighted in a recent study of novel T2D-associated SNPs in several populations of different ethnic origins [27]. In this context, further GWA studies in Arabic populations from Maghreb are necessary to further define the genetic components of T2D in these populations.
**Abbreviations**

BMI: Body Mass Index; CI: Confidence interval; K⁺-ATP channel: ATP-sensitive potassium channel; MAF: Minor Allele Frequency; OR: Odds ratio; PCR: Polymerase Chain Reaction; SNP: Single Nucleotide Polymorphism; T2D: Type 2 Diabetes.
Competing interests

The authors declare that there is no duality of interest.
Authors’ contributions

IE and NM participated in the design of the study, carried out the SNP genotyping and the analyses of the genotype data, and contributed to the statistical analyses and the drafting of the manuscript. SC contributed to the statistical analyses and participated in the writing of the manuscript. EV participated in the SNP genotyping and some of the genetic analyses. AD carried out some of the genotyping experiments. MC and MK coordinated the patients’ recruitment. WYA, PF and TM contributed to the manuscript editing. MV contributed to the design and coordination of the study, to the genetic analyses and drafted the manuscript. All authors read and approved the final manuscript.
Acknowledgments

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References


Table 1. Characteristics of the T2D patients and control subjects from the Tunisian population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n = 513)</th>
<th>T2D (n = 884)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male/Female)</td>
<td>258/255</td>
<td>406/478</td>
<td>0.115†</td>
</tr>
<tr>
<td>Age at examination (years)</td>
<td>60 ± 8.69</td>
<td>59.42 ± 11.09</td>
<td>0.312‡</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>24.83 ± 2.73</td>
<td>27.82 ± 5.30</td>
<td>4.81.10⁻³¹‡</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.12 ± 14.33</td>
<td>139.80 ± 28.13</td>
<td>7.62.10⁻²⁸‡</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.18 ± 10.55</td>
<td>80.92 ± 12.73</td>
<td>3.65.10⁻⁴‡</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.05 ± 0.64</td>
<td>12.67 ± 5.30</td>
<td>2.45.10⁻¹⁷²‡</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.47 ± 1.23</td>
<td>9.49 ± 3.89</td>
<td>2.33.10⁻⁴⁶‡</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.64 ± 1.28</td>
<td>5.26 ± 1.42</td>
<td>4.52.10⁻¹⁶‡</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.18 ± 0.60</td>
<td>1.77 ± 1.31</td>
<td>1.41.10⁻²¹‡</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.27 ± 0.39</td>
<td>1.07 ± 0.38</td>
<td>6.52.10⁻⁵‡</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.59 ± 1.60</td>
<td>3.77 ± 1.37</td>
<td>5.02.10⁻⁵‡</td>
</tr>
</tbody>
</table>

N: number of total subjects

Data are means ± SD.

†Chi square test (two-tailed).

‡Student’s t-test (two-tailed).
Table 2. T2D association for candidate SNPs in the Tunisian study sample of 1,397 individuals

<table>
<thead>
<tr>
<th>Gene SNP</th>
<th>Genotype</th>
<th>Control/T2D</th>
<th>Additive model</th>
<th>Dominant model</th>
<th>Recessive model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>OR (95% CI)</td>
<td>P †</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>TCF7L2</td>
<td></td>
<td>n 511/863</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs7903146</td>
<td>CC (1/1)</td>
<td>181/250</td>
<td>1.24 (0.95-1.61)</td>
<td>0.135</td>
<td>1.33 (1.04-1.70)</td>
</tr>
<tr>
<td></td>
<td>CT (1/2)</td>
<td>235/396</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT (2/2)</td>
<td>95/217</td>
<td>1.56 (1.13-2.16)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAF (T)</td>
<td>0.42/0.48</td>
<td>1.25 (1.06-1.47)</td>
<td>0.006†</td>
<td></td>
</tr>
<tr>
<td>KCNJ11</td>
<td></td>
<td>n 503/805</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs5219</td>
<td>EE (1/1)</td>
<td>250/371</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EK (1/2)</td>
<td>213/352</td>
<td>1.03 (0.80-1.31)</td>
<td>0.399</td>
<td>1.06 (0.84-1.34)</td>
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<tr>
<td></td>
<td>KK (2/2)</td>
<td>40/82</td>
<td>1.23 (0.80-1.90)</td>
<td>0.148</td>
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<tr>
<td></td>
<td>MAF (K)</td>
<td>0.29/0.32</td>
<td>1.07 (0.90-1.29)</td>
<td>0.440‡</td>
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<td>GCK</td>
<td></td>
<td>n 505/865</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>rs1799884</td>
<td>GG (1/1)</td>
<td>324/552</td>
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<tr>
<td></td>
<td>GA (1/2)</td>
<td>157/272</td>
<td>1.01 (0.79-1.30)</td>
<td>0.939</td>
<td>1.03 (0.81-1.32)</td>
</tr>
<tr>
<td></td>
<td>AA (2/2)</td>
<td>24/41</td>
<td>1.20 (0.69-2.08)</td>
<td>0.902</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAF (A)</td>
<td>0.20/0.20</td>
<td>1.04 (0.86-1.28)</td>
<td>0.640‡</td>
<td></td>
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<tr>
<td>HHEX</td>
<td></td>
<td>n 504/795</td>
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<tr>
<td>rs7923837</td>
<td>GG (1/1)</td>
<td>271/448</td>
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<tr>
<td></td>
<td>GA (1/2)</td>
<td>200/292</td>
<td>0.88 (0.69-1.13)</td>
<td>0.661</td>
<td>0.89 (0.70-1.13)</td>
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<tr>
<td></td>
<td>AA (2/2)</td>
<td>33/55</td>
<td>0.97 (0.60-1.58)</td>
<td>0.934</td>
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<tr>
<td></td>
<td>MAF (A)</td>
<td>0.26/0.25</td>
<td>0.93 (0.77-1.13)</td>
<td>0.470‡</td>
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<tr>
<td>ENPP1</td>
<td></td>
<td>n 499/809</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1044498</td>
<td>KK (1/1)</td>
<td>228/402</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>KQ (1/2)</td>
<td>205/311</td>
<td>0.86 (0.67-1.12)</td>
<td>0.243</td>
<td>0.84 (0.66-1.07)</td>
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<td>QQ (2/2)</td>
<td>66/96</td>
<td>0.78 (0.54-1.14)</td>
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<tr>
<td></td>
<td>MAF (Q)</td>
<td>0.33/0.31</td>
<td>0.88 (0.74-1.04)</td>
<td>0.140‡</td>
<td></td>
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</table>

n: number of total subjects, MAF: Minor allele frequency, †,‡,* Genotype specific P values and OR are adjusted for age gender and BMI in each additive, dominant or recessive genetic model, respectively.

#Allele-specific P values and OR of the log-additive genetic model are adjusted for age, gender and BMI.

1 Genetic additive model: 1/1 vs. 1/2, 2/2 genotypes
2 Genetic dominant model: 1/1 vs. 1/2 + 2/2 genotypes
3 Genetic recessive model: 1/1 + 1/2 vs. 2/2 genotypes
Additional files

Additional file 1
Format: doc
Title: supplementary table 1
Description: Hardy-Weinberg equilibrium for each studied SNP

Additional file 2
Format: doc
Title: supplementary table 2
Description: Minimum detectable effect size with a statistical power of 80%
Additional files provided with this submission:

Additional file 1: supplementary tables.doc, 46K
http://www.biomedcentral.com/imedia/2591586802340127/supp1.doc