Original article

Contribution of common variants of ENPP1, IGF2BP2, KCNJ11, MLXIPL, PPARγ, SLC30A8 and TCF7L2 to the risk of type 2 diabetes in Lebanese and Tunisian Arabs

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Abstract

Background. – While several type 2 diabetes mellitus (T2DM) susceptibility loci identified through genome-wide association studies (GWAS) have been replicated in many populations, their association in Arabs has not been reported. For this reason, the present study looked at the contribution of ENPP1 (rs1044498), IGF2BP2 (rs1470579), KCNJ11 (rs5219), MLXIPL (rs7800944), PPARγ (rs1801282), SLC30A8 (rs13266634) and TCF7L2 (rs7903146) SNPs to the risk of T2DM in Lebanese and Tunisian Arabs.

Methods. – Study subjects (case/controls) were Lebanese (751/918) and Tunisians (1470/838). Genotyping was carried out by the allelic discrimination method.

Results. – In Lebanese and Tunisians, neither ENPP1 nor MLXIPL was associated with T2DM, whereas TCF7L2 was significantly associated with an increased risk of T2DM in both the Lebanese [P < 0.001; OR (95% CI): 1.38 (1.20–1.59)] and Tunisians [P < 0.001; OR (95% CI): 1.36 (1.18–1.56)]. Differential associations of IGF2BP2, KCNJ11, PPARγ and SLC30A8 with T2DM were noted in the two populations. IGF2BP2 [P = 1.3 × 10⁻⁵; OR (95% CI): 1.66 (1.42–1.94)] and PPARγ [P = 0.005; OR (95% CI): 1.41 (1.10–1.80)] were associated with T2DM in the Lebanese, but not Tunisians, while KCNJ11 [P = 8.0 × 10⁻⁴; OR (95% CI): 1.27 (1.09–1.47)] and SLC30A8 [P = 1.6 × 10⁻⁵; OR (95% CI): 1.37 (1.15–1.62)] were associated with T2DM in the Tunisians, but not Lebanese, after adjusting for gender and body mass index.

Conclusion. – T2DM susceptibility loci SNPs identified through GWAS showed differential associations with T2DM in two Arab populations, thus further confirming the ethnic contributions of these variants to T2DM susceptibility.

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Keywords: Type 2 diabetes; Genetics; Lebanon; Tunisia; Arab population; Genome-wide association studies; SNPs; ENPP1; IGF2BP2; KCNJ11; MLXIPL; PPARγ; SLC30A8; TCF7L2

Résumé

Contribution de variants fréquents de ENPP1, IGF2BP2, KCNJ11, MLXIPL, PPARγ, SLC30A8 et TCF7L2 au risque de diabète de type 2 dans des populations arabes libanaise et tunisienne.

But. – Bien que plusieurs loci de susceptibilité au diabète de type 2 (DT2), identifiés par des études d’associations pangénomiques (GWAS), soient répliqués dans de nombreuses populations, leur association au DT2 dans les populations arabes n’a été pas signalée. Notre objectif était de tester la contribution des SNPs à risque au DT2, rs1044498 (ENPP1), rs1470579 (IGF2BP2), rs5219 (KCNJ11), rs7800944 (MLXIPL), rs1801282 (PPARγ), rs13266634 (SLC30A8), et rs7903146 (TCF7L2) dans deux populations arabes, libanaise et tunisienne.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease characterized by hyperglycaemia as a result of impaired insulin secretion, insulin resistance and increased hepatic glucose output [1,2]. Multiple environmental/lifestyle and genetic factors are involved in the onset and development of T2DM, and in determining T2DM phenotype [3,4]. A number of genes were reported to be associated with T2DM based on their relevance to T2DM pathogenesis [3,5]. However, this so-called "candidate gene" approach was limited in power to detect novel T2DM susceptibility genes, given the complex nature of the disease. This necessitated the search for novel genetic variants contributing to the risk of T2DM [3,6,7]. Genome-wide association studies (GWAS) using greater than 1,000,000 single nucleotide polymorphisms (SNPs) and high throughput technology can overcome the limitations of the candidate gene approach, leading to the identification of several SNPs that often have undefined functions [7].

Since 2007, GWAS have identified several susceptibility loci for T2DM. These included ENPP1, IGF2BP2, KCNJ11, MLXIPL, PPARγ, SLC30A8 and TCF7L2 [8–11], all reported to increase the risk of T2DM in Caucasians. Although these associations have since been replicated in non-Caucasian populations [12–14], the contribution of these, and most likely other, loci in Arab populations was not as clear. For example, a study in Morocco could find no significant associations between SNPs in CDKAL1, CDKN2B, HHEX, IGF2BP2 and SLC30A8 with T2DM [15], whereas an association between SNPs at the TCF7L2 locus, but not ENPP1, HHEX and KCNJ11, and T2DM was reported among Tunisians [16]. More recent studies in Lebanese Arabs also found no association with SNPs at the CDKNA2A/2B locus [11], but reported an association between T2DM and SNPs at the CDKAL1 [11] and IGF2BP2 [10] loci.

Present-day Arabs are a multiethnic population that ranges from the Arabian (Persian) Gulf to the Atlantic Ocean, with origins that can be classified according to their area of inhabitance (such as North Africa, the Arabian peninsula or Eastern Mediterranean). The present study has investigated the distribution of seven GWAS-confirmed T2DM susceptibility loci in Lebanese and Tunisian Arabs. Lebanon is located in the Eastern Mediterranean with a population that is an admixture of Ancient Phoenician pooled with Arabian and Western European lineages, brought about by the Islamic expansion from the Arabian Peninsula in the seventh century and Crusader activities in the 11th–13th centuries, respectively [17]. Tunisia is situated in North Africa, and the genetic makeup of its present-day inhabitants is the outcome of the partial admixture of its original (African) Berber population with the recognized (albeit still debatable) Phoenicians and Romans and, later, with Arab-speaking populations from the Arabian Peninsula, Turks (Ottoman Empire expansion to North Africa) and, more recently, Europeans (French) [18].

2. Subjects and methods

Table 1 presents the characteristics of the study subjects. The study used a case-control approach involving Lebanese and Tunisian T2DM patients and control subjects. The Lebanese participants comprised 751 T2DM patients who were recruited from the endocrinology outpatient clinics of Rizk Hospital, Rafic Hariri University Hospital and Saint Joseph Medical Center (Beirut, Lebanon), and the Tunisian subjects comprised 1470 patients recruited from the outpatients diabetes clinics of Farhat Hached Hospital (Sousse, Tunisia) and Hôpital Fattouma Bourguiba (Monastir, Tunisia). T2DM diagnosis was based on clinical and laboratory criteria, as per the 2003 Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, which were consistent with World Health Organization (WHO) criteria [19]. Exclusion criteria included other types of diabetes, autoimmune diseases, a positive anti-GAD, anti-IA2 or islet cell antibody (ICA) autoantibody response and diagnosis with T2DM before age 35 years. Diabetes treatment included diet and/or oral antidiabetic drugs (OADs) and/or insulin; all subjects requiring insulin had been treated with oral drugs for at least 2 years.

The control group included 918 Lebanese (546 men and 372 women) and 838 Tunisian (398 men and 440 women) normoglycaemic subjects (fasting plasma glucose <6.1 mmol/L and 2-h plasma glucose <7.8 mmol/L), with no known personal or family history of diabetes. Also, they were not related to anyone in either the control or study group, and had been drawn.
2.1. SNP genotyping

Total genomic DNA was isolated from the peripheral blood lymphocytes of the study subjects using DNA Blood Mini Kits (QIAGEN, Hilden, Germany), as per the manufacturer’s recommendations. SNPs in seven T2DM susceptibility loci, recently identified by GWAS, were genotyped, and included rs1044498 in ENPP1, rs1470579 in IGF2BP2, rs5219 in KCNJ11, rs7800944 in MLXIPL, rs1801282 in PPARγ, rs13266634 in SLC30A8 and rs7903146 in TCF7L2. Genotyping was performed by the allelic discrimination method (StepOne Real-Time PCR System, Applied Biosystems, Foster City, CA, USA), using commercially available Assay-on-Demand VIC- and FAM-labeled primers (Table 2), with well-defined genotype clusters. Genotype frequencies of the seven variants were consistent with the Hardy–Weinberg equilibrium (HWE) in both populations (Table 2), and the minor allele frequencies (MAFs) obtained were consistent with those in the HapMap CEU sample. Replicate blinded quality-control samples were included to assess reproducibility of the genotyping procedure; the concordance was greater than 99%.

2.2. Statistical analyses

Data were expressed as means±SD (continuous variables) and as percentages of the total (categorical variables), while intergroup significance was assessed by Student’s t test (continuous variables) and χ² test (categorical variables). Allele frequencies were calculated by the gene-counting method; each SNP was tested for HWE by the χ² goodness-of-fit test using HPlus 2.5 software (http://cdsweb01.fhcrc.org/HPlus). The genotype

Table 1
Clinical characteristics of the study participants.

<table>
<thead>
<tr>
<th>Gender (male:female)</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lebanese</td>
<td>333:418</td>
<td>546:372</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>59.9±12.3</td>
<td>59.3±12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>28.4±4.9</td>
<td>23.4±3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>132.4±17.1</td>
<td>121.3±14.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.9±14.2</td>
<td>79.2±11.2</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>10.3±3.8</td>
<td>5.3±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.9±3.1</td>
<td>4.9±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age of T2DM onset (years)</td>
<td>52.2±12.3</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>T2DM duration (years)</td>
<td>9.0±6.4</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Genotyping assay: the single nucleotide polymorphisms (SNPs) analyzed.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Chromosome</th>
<th>Assay identification</th>
<th>Lebanese (HWE)</th>
<th>Power (%)</th>
<th>Tunisians (HWE)</th>
<th>Power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENPP1</td>
<td>rs1044498</td>
<td>6</td>
<td>C_1207994_20</td>
<td>0.49</td>
<td>65</td>
<td>0.62</td>
<td>92</td>
</tr>
<tr>
<td>IGF2BP2</td>
<td>rs1470579</td>
<td>3</td>
<td>C_2165184_10</td>
<td>0.16</td>
<td>100</td>
<td>0.15</td>
<td>71</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>rs5219</td>
<td>11</td>
<td>C_11654065_10</td>
<td>0.18</td>
<td>55</td>
<td>0.32</td>
<td>75</td>
</tr>
<tr>
<td>MLXIPL</td>
<td>rs7800944</td>
<td>7</td>
<td>C_2632484_14</td>
<td>0.92</td>
<td>63</td>
<td>0.67</td>
<td>96</td>
</tr>
<tr>
<td>PPARγ</td>
<td>rs1801282</td>
<td>3</td>
<td>C_1129864_14</td>
<td>0.81</td>
<td>84</td>
<td>0.39</td>
<td>89</td>
</tr>
<tr>
<td>SLC30A8</td>
<td>rs13266634</td>
<td>8</td>
<td>C_357888_10</td>
<td>0.85</td>
<td>98</td>
<td>0.32</td>
<td>84</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs7903146</td>
<td>10</td>
<td>C_29347861_10</td>
<td>0.56</td>
<td>83</td>
<td>0.20</td>
<td>77</td>
</tr>
</tbody>
</table>

HWE: Hardy–Weinberg equilibrium P value, determined by χ² goodness-of-fit test.

a Seven hundred and fifty-one T2DM patients and 918 controls.
b One thousand four hundred and seventy T2DM patients and 838 normoglycaemic controls.
c Analyzed by CaTS power calculator.
association of each SNP with T2DM susceptibility was tested by $\chi^2$ statistics with two degrees of freedom, and the covariates controlled for were gender, age and body mass index (BMI). Overall power was determined using the CaTS power calculator (http://www.sph.umich.edu/csg/abecasis/CaTS). Logistic-regression analysis was performed to calculate the risk allele-specific values, odds ratios (ORs) and 95% confidence intervals (CIs).

### 3. Results

#### 3.1. Study subjects

The clinical characteristics of the two study populations are shown in Table 1. Lebanon and Tunisia had comparable T2DM (13.1% and 12.6%, respectively) and impaired glucose tolerance (9.0% and 10.0%, respectively) prevalence rates. HbA1c levels were higher among Tunisians (9.7%) than among Lebanese (8.9%; $P = 0.047$). Lebanese and Tunisian participants were all of comparable age and BMI distributions among both patients and controls. While age at T2DM onset was higher among the Lebanese, longer T2DM durations were noted in the Tunisian population.

#### 3.2. Allelic association

Table 2 presents the seven genotyped SNPs and the test results for HWE deviation. The overall power (calculated as the average power of the seven SNPs analyzed) was 78.3% for the Lebanese and 83.4% for the Tunisians. No deviation from HWE was observed for the seven SNPs in the two populations analyzed. Table 3 shows the association between the tested SNPs and T2DM risk after controlling for gender and BMI. In both Lebanese and Tunisians, ENPP1 rs1044498 ($P = 0.231$ and $P = 0.939$, respectively) and MLXIPL ($P = 0.284$ and $P = 0.984$, respectively) were not associated with T2DM. In contrast, TCF7L2 rs7903146 was markedly associated with T2DM in both the Lebanese [$P = 4.6 \times 10^{-6}$, OR (95% CI): 1.41 (1.10–1.80)] and Tunisians [$P = 6.0 \times 10^{-6}$, OR (95% CI): 1.37 (1.15–1.62)]. In addition, IGF2BP2 rs1470579, KCNJ11 rs5219, PPARγ rs1801282 and SLC30A8 displayed significant associations with T2DM in both Tunisians, but not in the Lebanese.

### 4. Discussion

The present study looked at the differential contributions of T2DM susceptibility loci identified through GWAS in two Arab communities. Previously, we reported on the
association of variants in the CDKAL1 [11] and IGF2BP2 [10] genes with T2DM in Lebanese, and on the association of TCF7L2 rs7903146 with T2DM in Tunisians [16]. This list of T2DM-associated variants was expanded by examining the associations of ENPP1 rs1044498, IGF2BP2 rs1470579, KCNJ11 rs5219, MLXIPL rs7800944, PPARY rs1801282, SLC30A8 rs13266634 and TCF7L2 rs7903146, among the few variants first identified by GWAS in Caucasians. Consistent with the differences in the ethnic origins of Lebanese and Tunisians [17,18], there was a statistically significant association with TCF7L2 rs7903146, and no associations at the ENPP1 and MLXIPL loci, in both communities, and a variable association with T2DM in either population at the remaining four loci, thereby demonstrating that the association of these SNPs with T2DM among Arabs needs to be discussed in the context of racial/ethnic backgrounds [5].

Such a varied distribution of these variants was noted among both Lebanese and Tunisians. In the control subjects, the MAFs of IGF2BP2 rs1470579, MLXIPL rs7800944 and TCF7L2 rs7903146 were similar among the Lebanese, but higher in Tunisians compared with the frequencies established for Caucasians. In addition, the MAF of PPARY rs1801282 was comparable, while that of ENPP1 rs1044498 was higher and that of KCNJ11 rs5219 lower in both Lebanese and Tunisians compared with Caucasians. This confirms the ethnic diversity in the distribution of these causal variants found by GWAS among populations of Caucasian descent, including Arab-speaking populations, which may also explain the apparent ethnicity-related health disparities associated with these variants.

Despite the fact that both our Lebanese and Tunisian samples were sufficiently powered, no significant association was detected between ENPP1 (K121Q) or MLXIPL and T2DM, each an initially T2DM-associated locus identified through GWAS. A recent meta-analysis of 11,855 Chinese subjects reported a higher association [20,21]. Our present study found that ENPP1 was not associated with T2DM in either population, thereby in agreement with studies in Chinese [20,21], Iranian [22] and North Indian [23] populations, but in sharp contrast to earlier studies in Tunisians [24] and Moroccans [9], in which ENPP1 was linked to an increased T2DM risk. In addition to the small number of subjects in the Tunisian (110 cases, 261 controls) and Moroccan (503 cases, 412 controls) studies, the failure to control for covariates most likely had an impact on the contributions of any potential susceptibility loci to the overall T2DM risk. This suggests that the contributions of these loci may be either population-specific or perhaps false positives stemming from the small sample sizes that characterized the first wave of GWAS, thereby questioning the validity of the conclusions reached in those studies.

The MLXIPL interacting protein-like (MLXIPL) gene—also known as the “carbohydrate-responsive element-binding protein (ChREBP) gene”—encodes a helix-loop-helix leucine zipper transcription factor. ChREBP binds to and activates carbohydrate-response elements found in the promoter regions of genes involved in triglyceride synthesis [25], and its expression in human adipose tissue predicts insulin sensitivity, indicating that it may be an effective target for treating diabetes. MLXIPL rs7800944 was previously associated with T2DM in a Japanese population [26], but did not contribute to altered T2DM risk in our Lebanese and Tunisian populations, in contrast to TCF7L2 rs7903146, which was strongly associated with T2DM in both these populations. In this context, any discordance of association noted between our Lebanese and Tunisian groups, highlighted by the calculated ORs (95% CI) in Tunisians [1.36 (1.18–1.56)] and Lebanese [1.38 (1.20–1.59)], was mainly due to gene–environment interactions and was subtle compared with the other studied loci, thereby further establishing TCF7L2 variants (including rs7903146) as some of the strongest T2DM susceptibility loci studied thus far [27,28].

Of interest were the selective associations of KCNJ11 rs5219 and SLC30A8 rs13266634 with T2DM in Tunisians only, whereas IGF2BP2 rs1470579 and PPARY rs1801282 were associated with T2DM in Lebanese, but not Tunisian, subjects. However, the apparent failure to detect statistically significant associations for KCNJ11 and SLC30A8 SNPs in the Lebanese, and IGF2BP2 and PPARY variants in Tunisians, was not due to the power of the studies for detecting these associations, as at least an 80% power (based on the calculated allele frequencies and the available sample sizes) was attained for detecting these associations. Except for ENPP1, for which the MAFs were higher in Lebanese (0.217) and in Tunisians (0.331) compared with Europeans (0.135), the MAFs of the remaining loci were generally comparable between our present study populations and European populations. This indicates that the variable association of IGF2BP2, KCNJ11, PPARY and SLC30A8 SNPs in Lebanese and Tunisian Arabs cannot be explained by effect sizes [29,30], but rather by allelic heterogeneity between the two populations [29,30]. As such, the differential association of these variants cannot be attributed simply to chance, and the associations for many of the variants identified in European and Asian populations also appear to be relevant in Arab populations [29,31].

In conclusion, our present study has demonstrated that the common variants associated with T2DM, as identified through GWAS in populations of European ancestry and subsequently replicated in other populations, are selectively associated with T2DM in Lebanese and Tunisian Arabs. While the failure to detect associations in some loci remains debatable, the assessment of multietnic groups, as done here with Lebanese and Tunisian Arabs, is of paramount importance in the identification of genetic markers of T2DM. Nevertheless, the functional implications of these genetic associations remain to be seen, as these and other related loci represent surrogate markers that may not necessarily contribute to the genetic aetiology of T2DM.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.
References


