Abstract

Aims. – Recent genome-wide association studies (GWAS) and previous approaches have identified many genetic variants associated with type 2 diabetes (T2D) in populations of European descent, but their contribution in Arab populations from North Africa is unknown. Our study aimed to validate these markers and to assess their combined effects, using large case-control studies of Moroccan and Tunisian individuals.

Methods. – Overall, 44 polymorphisms, located at 37 validated European loci, were first analyzed in 1055 normoglycaemic controls and 1193 T2D cases from Morocco. Associations and trends were then assessed in 942 normoglycaemic controls and 1446 T2D cases from Tunisia. Finally, their ability to discriminate cases from controls was evaluated.

Results. – Carrying a genetic variant in BCL11A, ADAMTS9, IGF2BP2, WFS1, CDKAL1, TP53INP1, CDKN2A/B, TCF7L2, KCNQ1, HNF1A, FTO, MC4R and GCK increased the risk of T2D when assessing the Moroccan and Tunisian samples together. Each additional risk allele increased the susceptibility for developing the disease by 12% ($P = 9.0 \times 10^{-9}$). Genotype information for 13 polymorphisms slightly improved the classification of North Africans with and without T2D, as assessed by clinical parameters, with an increase in the area under the receiver operating characteristic curve from 0.64 to 0.67 ($P = 0.004$).

Conclusion. – In addition to TCF7L2, 12 additional loci were found to be shared between Europeans and North African Arabs. As for Europeans, the reliability of genetic testing based on these markers to determine the risk for T2D is low. More genome-wide studies, including next-generation sequencing, in North African populations are needed to identify the genetic variants responsible for ethnic disparities in T2D susceptibility.

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Keywords: Polymorphism; Type 2 diabetes; Morocco; Tunisia

Résumé

Polymorphismes génétiques associés au diabète de type 2 chez les européens et les populations nord-africaines.

Objectifs. – Les récentes études d’association sur le génome entier et les approches précédentes ont permis d’identifier de nombreux polymorphismes associés au diabète de type 2 (DT2) dans des populations d’origine européenne mais leur contribution dans des populations nord-africaines reste inconnue. Notre but était donc de valider ces marqueurs et d’évaluer leurs effets combinés en utilisant des larges études cas–témoin chez des individus marocains et tunisiens.

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1. Introduction

The recent adoption of sedentary Western lifestyles has led to an increased prevalence of type 2 diabetes (T2D) in North African countries such as Morocco and Tunisia (7% and 9%, respectively). However, in these populations, the genetic predisposition to develop the disorder remains unclear. It has been estimated that 70% of Tunisian T2D patients have a positive family history of the disease involving at least one parent, as well as siblings, uncles and aunts on both sides [1]. In Moroccans and Tunisians, the association with T2D was analyzed for the limited number of polymorphisms previously identified in European studies. Although most associations were not convincingly confirmed due to the small number of samples and modest effect sizes, the TCF7L2 rs7903146 polymorphism was clearly identified as a genetic risk factor [2,3].

Since 2007, genome-wide association studies (GWAS) and their meta-analyses have produced numerous successes in identifying genetic variants that explain some of the interindividual variations observed in T2D susceptibility [4]. However, with few exceptions, GWAS have been centered on populations of European descent, and the degree to which the knowledge gained from these studies is transferable to other populations has not been extensively investigated.

In the present study, 44 markers located at 37 genetic regions and validated in previous European studies were first analyzed in 1055 normoglycaemic controls and 1193 T2D cases from Morocco. The associations and trends were then assessed in 942 normoglycaemic controls and 1446 T2D cases from Tunisia. Finally, their combined effects and ability to discriminate cases from controls were evaluated.

2. Research design and methods

2.1. Subjects

The study participants’ main clinical characteristics are presented in Supplementary data, Table S1. All subjects were of Arab descent. Unrelated T2D patients from Morocco were recruited from diabetes associations and health centres in five different regions of the country (Fez, Sale, Taounate, Sefrou and Taza), while those from Tunisia were recruited from outpatient endocrinology clinics in southern, central and northern parts of the country by treating physicians. T2D diagnosis was based on fasting plasma glucose greater or equal to 7 mmol/L and/or treatment with glucose-lowering agents. In Morocco, control subjects were recruited from an unselected population undergoing routine health checkups at the same health centres. In Tunisia, blood donors, healthy volunteers and hospital/university staff members from the same areas of the country were matched with T2D patients for age and gender. Control individuals had to meet the following inclusion criteria: greater or equal to 40 years of age; normoglycaemia (fasting glucose <5.6 mmol/L); no regular medication (including weight-loss diet) within 6 months of entering the study; and no personal or first-degree history of diabetes.

The study was approved by the relevant local ethics committees, with informed consent obtained from each participant. Clinical characteristics of the Moroccan and Tunisian sample populations are reported in Supplementary data, Table S1.

2.2. Genotyping

In Moroccans, the genotyping of polymorphisms was performed using the SNPlex™ technology, based on oligonucleotide ligation assay (OLA) combined with multiplex polymerase chain reaction (PCR) target amplification (Applied Biosystems, Foster City, CA, USA). Four SNPs (rs1260326, rs11708067, rs340874 and rs2191349) failed to pass genotyping quality-control criteria: individual SNP call rates greater or equal to 95%; and Hardy–Weinberg equilibrium in cases and control samples ($P \geq 0.01$). Allelic discrimination was performed by capillary electrophoresis analysis using a 3730xl DNA Analyzer (Applied Biosystems) and GeneMapper3.7 software. All polymorphisms showing associations or trends ($P \leq 0.1$) in at least one of the three genetic models (log-additive, recessive and dominant) were then genotyped in our Tunisian samples, using an allelic discrimination assay-by-design TaqMan method on a 7900HT Fast Real-Time PCR System (Applied Biosystems).
2.3. Statistical analyses

Odds ratios (ORs) were calculated using logistic-regression models adjusted for either age, body mass index (BMI) and gender or age and gender (for polymorphisms having a known association with T2D through their effect on BMI). An association was considered when: it was observed in both Moroccan and Tunisian samples; and the risk allele was no different from that reported in Europeans. Given that all polymorphisms had previously been validated as genetic risk factors in populations of European descent, and that most of them were also associated with T2D in multiple ethnic groups, it was considered unlikely to produce false-positive results. For this reason, multiple testing was not applied and a two-sided P-value threshold of 0.05 was adopted. The statistical power to detect an association with T2D was calculated retrospectively, based on the number of cases and controls for each association study, the risk allele frequency for each polymorphism and the effect size observed in either North Africans or Europeans (Supplementary data, Fig. S1 and Tables S2 and S3). Fixed- or random-effects (in cases of heterogeneity only) meta-analyses were performed, using the “rmeta” package, to provide a pooled OR with a 95% confidence interval (CI). The Woolf test was applied to test genotypic heterogeneity between Tunisians and Moroccans. Also explored was the effect of multiple polymorphisms, using a logistic-regression model including a variable for the number of risk alleles to quantify the risk per supplementary allele for all variants included in the model. In addition, ORs corresponding to a given number of risk alleles compared with the reference group were calculated. The ability of this model to discriminate between normoglycaemic and T2D subjects was evaluated through receiver operating characteristic (ROC) curves, using logistic-regression models including genetic and/or clinical parameters. The area under the ROC curve (AUROC) was calculated as a measure of discriminative accuracy. The method of Hanley and McNeil was used for calculation of the difference between two AUROCs, while the QUANTO programme (http://hydra.usc.edu/GxE/) was used for power calculations. Pair-wise linkage disequilibrium between genetic markers was assessed using the R genetics package. All P-values were two-sided, and R statistics (version 2.5.1) software was used for the general statistical analyses.

3. Results

3.1. Association with T2D in Moroccans

In the present study, most genetic markers were tag polymorphisms from GWAS for which the aetiological variant(s) was/were unknown. For other markers, the best inheritance models were mostly based on individuals of European origin. Thus, genotype distribution was analyzed in 1055 normoglycaemic controls and 1193 T2D cases while assuming three models of inheritance: dominant; recessive; and log-additive. An association trend was considered if, in at least one model, the statistical significance reached a P-value less or equal to 0.1. Of the 44 polymorphisms, 29 were not associated with T2D in Moroccans (Supplementary data, Tables S1 and S4). However, carrying one of the 15 genetic variants in BCL11A, ADAMTS9, IGF2BP2, WFS1, CDKN2A/B, TP53INP1, TCF7L2, KCNQ1, HNF1A, FTO, MC4R and GCK increased the risk of T2D in our sample populations (Table 1). In Moroccans, a statistical power greater than 80% for all genetic models of inheritance was
### Table 1
European risk loci at which at least one polymorphism tended to be associated with type 2 diabetes in North African samples.

<table>
<thead>
<tr>
<th>Locus</th>
<th>rs ID</th>
<th>LD CEU</th>
<th>LD M/T</th>
<th>Risk allele frequency (%)</th>
<th>Odds ratio (95 % CI)</th>
<th>Log-additive P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HapMap (CEU)</td>
<td>North Africans</td>
<td>Controls</td>
<td>Cases</td>
<td>Dominant</td>
</tr>
<tr>
<td>BCL11A</td>
<td>rs243021</td>
<td>A 45.8</td>
<td>M 55.7</td>
<td>57.4</td>
<td>0.93 (0.74–1.18)</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T 52.8</td>
<td>M 57.6</td>
<td>1.20 (0.94–1.54)</td>
<td>0.13</td>
<td>1.27 (1.02–1.56)</td>
</tr>
<tr>
<td>ADAMTS9</td>
<td>rs6795735</td>
<td>C 54.2</td>
<td>M 31.8</td>
<td>32.3</td>
<td>1.07 (0.89–1.29)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>rs4607103</td>
<td>C 80.8</td>
<td>M 62.6</td>
<td>64.7</td>
<td>1.10 (0.84–1.43)</td>
<td>0.5</td>
</tr>
<tr>
<td>IGF2BP2</td>
<td>rs1470579</td>
<td>C 29.2</td>
<td>M 42.4</td>
<td>46.4</td>
<td>1.23 (1.01–1.50)</td>
<td>0.04</td>
</tr>
<tr>
<td>WFS1</td>
<td>rs10010131</td>
<td>G 73.3</td>
<td>M 69.6</td>
<td>72.6</td>
<td>1.37 (0.96–1.96)</td>
<td>0.09</td>
</tr>
<tr>
<td>CDKALI</td>
<td>rs7756992</td>
<td>G 25.0</td>
<td>M 31.1</td>
<td>33.2</td>
<td>1.09 (0.91–1.30)</td>
<td>0.37</td>
</tr>
<tr>
<td>TP53INP1</td>
<td>rs896854</td>
<td>T 47.5</td>
<td>M 53.1</td>
<td>56</td>
<td>1.09 (0.87–1.37)</td>
<td>0.45</td>
</tr>
<tr>
<td>CDKN2A/Brs10811661</td>
<td>M 0.07</td>
<td>T 79.2</td>
<td>M 82.4</td>
<td>84.6</td>
<td>1.30 (0.76–2.22)</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>rs64398</td>
<td>T 62.5</td>
<td>M 77.7</td>
<td>79.9</td>
<td>1.33 (0.86–2.04)</td>
<td>0.20</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs7903146</td>
<td>T 27.9</td>
<td>M 38.5</td>
<td>47.6</td>
<td>1.68 (1.39–2.03)</td>
<td>3.57 × 10⁻⁸</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>rs231362</td>
<td>G 52.0</td>
<td>M 53.2</td>
<td>54.9</td>
<td>1.23 (0.98–1.54)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>rs2283228</td>
<td>A 92.5</td>
<td>M 94.6</td>
<td>95.1</td>
<td>1.72 (0.32–9.09)</td>
<td>0.53</td>
</tr>
<tr>
<td>HNF1A</td>
<td>rs7957197</td>
<td>T 85</td>
<td>M 81.6</td>
<td>85.1</td>
<td>1.72 (0.99–2.94)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>rs1169288</td>
<td>G 28.3</td>
<td>M 58.7</td>
<td>60.6</td>
<td>0.95 (0.74–1.22)</td>
<td>0.69</td>
</tr>
<tr>
<td>FTO</td>
<td>rs1421085</td>
<td>C 44.8</td>
<td>M 35.6</td>
<td>39.5</td>
<td>1.24 (1.03–1.49)</td>
<td>0.02</td>
</tr>
<tr>
<td>MC4R</td>
<td>rs17782313</td>
<td>C 28.3</td>
<td>M 16.9</td>
<td>19.5</td>
<td>1.18 (0.97–1.43)</td>
<td>0.10</td>
</tr>
<tr>
<td>GCK</td>
<td>rs1799884</td>
<td>A 20.3</td>
<td>M 18.1</td>
<td>20.6</td>
<td>1.24 (1.02–1.50)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Odds ratios were adjusted for age, gender and body mass index (BMI), except for for a (adjusted for age and gender); association trends (P ≤ 0.1) are shown in boldface; M: Moroccans; T: Tunisians; na: not available.
observed only for the TCF7L2 genetic variant (Supplementary data, Tables S2 and S3). The FTO and MC4R polymorphisms were not associated with T2D when adjusted for BMI.

3.2. Confirmation in Tuniensians and meta-analysis

All 15 polymorphisms associated with T2D in the Moroccan samples were subsequently genotyped in 942 normoglycaemic controls and 1446 T2D cases from Tunisia. Overall, 10 polymorphisms located in the BCL11A, ADAMTS9, IGF2BP2, WFS1, CDKAL1, TP53INP1, CDKN2A/B, TCF7L2, KCNQ1 and HNF1A genes were also associated with T2D risk in this sample population. However, in Tuniensians, none of the genetic variants reached the statistical power of 80% to detect an association with T2D for all genetic models of inheritance (Supplementary data, Tables S2). For each polymorphism and each model of inheritance, no significant heterogeneity was detected between Tunisian and Moroccan ORs (Woolf test: P > 0.05) except for the TCF7L2 rs7903146 polymorphism (Woolf test: P = 0.02 on testing the log-additive model).

In addition, a meta-analysis was performed to provide pooled allelic ORs for each genetic variant (Fig. 1). Only two risk alleles were found to be not associated with T2D on assessing the Moroccan and Tunisian populations together: CDKN2A/B rs564398 (OR: 1.12 [0.99–1.26]; P = 0.06); and HNF1A rs1169288 (OR: 1.07 [0.97–1.17]; P = 0.19). When Moroccans and Tuniensians were analyzed together, there was sufficient power (using effect sizes as either reported in Europeans or observed in North Africans) to detect those allelic associations with T2D risk, which did not include the TP53INP1 and HNF1A genetic variants (Fig. 1).

3.3. Effects in Moroccans and Tuniensians combined

The 13 polymorphisms individually associated with T2D in the 1997 normoglycaemic controls and 2639 T2D cases from both Morocco and Tunisia were analyzed in combination. The percentage distribution of T2D and normoglycaemic individuals carrying increasing numbers of risk alleles is presented in Supplementary data, Fig. S1. In addition, the allelic ORs for T2D were calculated in comparison to the reference group carrying 0 to 10 risk alleles, representing 14.3% of the North African samples (Fig. 2). It was estimated that each additional risk allele increased the risk of T2D by 12% (P = 9.0 × 10−9). On average, individuals carrying at least 16 risk alleles (13.8% of the normoglycaemic controls and 20.3% of the T2D cases) were found to be 2.5 times more likely to develop the disorder compared with the reference group. The power to discriminate between T2D cases and normoglycaemic controls, using 13 genetic variants validated in North Africans, clinical parameters (BMI, age and gender) or both clinical and genetic factors, was evaluated by the AUROC curve: this was 0.64 for clinical parameters (BMI, age and gender); 0.60 for genetic markers; and 0.67 for both clinical and genetic factors (Fig. 3). Including genetic information in the model based on clinical factors significantly improved classification (P = 0.004).
risk difference may be amplified by gene–environment interactions [5]. Efforts are therefore necessary to determine whether the genetic susceptibility to develop T2D is similar or different across diverse ethnic groups.

Recently, Waters et al. [6] provided strong support for the idea of commonly shared genetic variations contributing to T2D risk in multiple populations, thereby questioning the “common SNP, rare mutation” model proposed by Dickson et al. [7]. Polymorphisms at 13 European loci were found to be also associated with T2D on analyzing Moroccans and Tunisians together (TCF7L2, HNF1A, CDKN2A/2B, IGF2BP2, WFS1, ADAMTS9, CDKAL1, MC4R, GCK, BCL11A, KCNJ1, TP53INP1 and FTO). Each additional risk allele significantly increased the susceptibility to develop T2D \( (P = 9.0 \times 10^{-9}) \), which is consistent with that reported in Europeans [8]. Also, no heterogeneity in genotype distribution was observed between Moroccans and Tunisians except for the TCF7L2 genetic variant, although the two CIs overlapped \((1.47[1.29–1.66]\) vs. \(1.19[1.04–1.37]\), respectively). Effect sizes for the TCF7L2 polymorphism previously reported in both Moroccans [2] and Tunisians [3] did not differ compared with the observations made in our present study (Woolf tests: \(P > 0.05\)). Thus, heterogeneity was probably due to a lack of sufficient power within each cohort.

Interestingly, allele frequencies, as well as the size and direction of their effect estimates, were comparable between our Arab populations and those of Europeans. Previous studies reported that the geographically closest populations might serve as good genetic references. Specifically, the HapMap CEU population (Utah residents with Northern and Western European ancestry) may provide evidence of relatively acceptable portability across North African populations [9]. Furthermore, analyses of mitochondrial DNA have shown that North Africans and Eurasians share common haplogroups [10].

As for Europeans, the reliability of genetic testing (based on 13 markers) for classifying North African Arabs with and without disease is low. However, such genetic information significantly improved the classification model based on clinical factors (net reclassification improvement: 30.3% [23.6–37.0]). Common variants with small effects or fewer rare variants with stronger effects have yet to be discovered, and might further improve discriminative accuracy. Furthermore, additional polymorphisms that affect diabetogenic traits or obesity, but which are not conventionally considered to be diabetes-predisposing loci, might also boost the effectiveness of genetic testing for T2D [11]. Moreover, our present study may have underestimated the impact of these genetic factors, given that our sample populations were not totally representative of the North African Arab populations. In fact, all of our study participants were unrelated (as confirmed by Hardy–Weinberg equilibrium for all genetic variants), whereas the prevalence of consanguinity in Morocco and Tunisia has been estimated to range from 15% to 25% [12,13], with most consanguineous marriages involving first cousins. In addition to increasing the risk for recessive disorders, this tradition might also explain, in part, the rapid increase in complex diseases.

Functional studies have observed that the majority of genetic variants associated with T2D in North African Arabs have potential effects on pancreatic beta-cell dysfunction [14]. Previous heritability analyses have suggested a stronger genetic contribution to components of insulin secretion or beta-cell function compared with insulin resistance, which may partly explain the lack of insulin resistance loci [15]. In North Africans, it was also observed that two loci, FTO and MC4R, were associated with T2D through their effects on BMI. In Europeans, GWAS have detected that FTO polymorphisms increase the risk of T2D [16–19]. However, their association with BMI has been questioned in Sub-Saharan Africans [20,21], suggesting that North African Arabs are genetically closer to Europeans. As for North Africans, different studies have reported an association between MC4R polymorphisms and T2D [22–24]. Therefore, this genetic marker of obesity needs to be considered when assessing the risk of T2D, even though this locus has never reached the threshold for genome-wide significance.

An association with T2D was not observed for genetic variants at 24 European loci. Given their low effect size, this may have been due to power issues (Supplementary data, Table S3). The two-stage design of our present study was driven by the fact that multiplexed genotyping was not successful in our Tunisian samples and that the amounts of DNA were limited for this population. Another possibility is that the linkage disequilibrium structure of each loci that failed to be associated was locally different compared with that of Europeans. In line with this hypothesis, the linkage disequilibrium between polymorphisms associated with T2D risk was comparable between North African Arabs and Europeans (HapMap CEU, NCBI build 36, dbSNP h126), which was not the case for genetic variants at loci not associated with T2D in North African Arabs. However, all of these polymorphisms are only markers of putative risk and not necessarily the functional genetic variants per se [25]. Therefore, an association between other polymorphisms and T2D cannot be excluded for those loci.

Although clinical features of T2D vary substantially across different ethnic groups, population differences at genetic-risk loci with significant genome-wide support remain poorly defined. Extending the HapMap (haplotype map of the human genome) to include other ethnic groups such as North Africans may be necessary to design specific arrays and to detect novel associations in genome-wide studies. Furthermore, separate populations are more likely to differ in their collection of rare alleles than in their collection of common alleles, as rare variants are usually more recent in origin [26]. However, recent studies have identified common polymorphisms that were specifically associated with T2D in Asian populations [27–32]. Thus, uncovering the role of rare and common variants in T2D through whole-genome sequencing and GWAS may be a necessary step towards understanding ethnic disparities in disease susceptibility.

In conclusion, the association between 13 polymorphisms and T2D in populations of European descent was also observed in North Africans. However, in Europeans, the discriminative accuracy of genetic testing based on these markers is low. Although a large number of samples were analyzed, our present
results need to be confirmed in other North African Arab populations to increase their statistical power.

Author contributions

S.C., I.E., Y.E.A., N.M., D.S. and C.N. researched data, contributed discussion, wrote manuscript, reviewed/edited manuscript.

L.C., M.V., T.M. and M.C. contributed discussion, reviewed/edited manuscript.

Y.L., L.Y. and D.B. researched data, reviewed/edited manuscript.

P.F. contributed discussion, wrote manuscript, reviewed/edited manuscript.

Disclosure of interest

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

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Appendix A. Supplementary data

Supplementary data (Fig. S1 and Tables S1–S4) associated with this article can be found at http://www.sciencedirect.com and in the online version at doi:10.1016/j.diabet.2012.02.003.

References


