Original article

Lack of replication of common EXT2 gene variants with susceptibility to type 2 diabetes in Lebanese Arabs

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Abstract

Objective. – Recent genome-wide association studies and replication analyses have reported the association of variants of the exostosin-2 (EXT2) gene and risk of type 2 diabetes mellitus (T2DM) in some populations, but not in others. This study investigated the associations of EXT2 variants rs1113132, rs3740878 and rs11037909 with T2DM in a Lebanese Arab population.

Methods. – This case-control study involved 995 T2DM patients and 1076 control subjects. Genotyping was done by the allelic exclusion method.

Results. – While minor allele frequencies (MAFs) of rs11037909 (P = 0.028) and rs3740878 (P = 0.048), but not rs1113132 (P = 0.841), were higher in patients, this was lost after correcting for multiple testing. Apart from EXT2 rs1113132, which was marginally associated with T2DM in the additive model (P = 0.054), but not after adjustment for covariates, none of the tested EXT2 SNPs were associated with T2DM in any of the genetic models tested. However, variable associations of EXT2 variants with T2DM were noted according to BMI status. While the three tested EXT2 variants were not associated with T2DM in obese subjects, rs1113132 and rs11037909, but not rs3740878, were associated with T2DM in non-obese subjects. Meta-analysis revealed a significant association of rs11037909 and a marginal association of rs3740878 with T2DM in the fixed model. Using a common (GTA) haplotype as reference, three-locus (rs1113132/rs11037909/rs3740878) haplotype analysis demonstrated no association between any of the EXT2 haplotypes with T2DM, not even before correcting for multiple testing.

Conclusion. – This study demonstrated no association of rs1113132, rs3740878 and rs11037909 EXT2 variants with T2DM.

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Keywords: Diabetes; Exostosin-2; Haplotypes; Replication studies

Résumé

Absence d’association entre les variants du gène EXT2 et le risque de diabète de type 2 chez les Arabes libanais.

Objectifs. – Des études récentes du génome entier GWA et des analyses de réplication dans certaines populations ont rapporté une association significative entre les variants du gène exostine (EXT2) et le risque du diabète type 2 (DMT2). L’objectif de ce travail est d’évaluer dans la population arabe libanaise la contribution des variants rs1113132, rs3740878 et rs11037909 du gène EXT2 au risque du diabète type 2.

Méthodes. – Cette étude cas-témoin porte sur 995 patients diabétiques de type 2 et 1076 sujets témoins. Le génotypage de ces polymorphismes est réalisé par la technique d’exclusion allélique.

Résultats. – Bien que nos résultats aient montré que les fréquences de l’allèle mineur (MAF) de rs11037909 (P = 0.028) et rs3740878 (P = 0.048), mais pas rs1113132 (P = 0.841), sont significativement plus élevées chez les patientes par rapport aux sujets témoins, cette significativité a été perdue après l’application de la correction de Bonferroni pour de multiples tests. À part le variant rs1113132 du gène EXT2, qui n’était que marginalement associé au DMT2 sous le modèle additif (P = 0.054), et qui a perdu cette significativité après ajustement selon des covariables potentielles, aucun des autres single nucleotide polymorphisms (SNP) étudiés n’était associé au risque du DMT2 sous les différents modèles génétiques. L’association des variants du gène EXT2 au risque du DMT2 a été notée selon l’IMC. Cependant, les trois variants de l’EXT2 n’ont pas été associés avec diabète de type 2 chez les sujets obèses, en revanche seulement le rs3740878 a été associé au DMT2 chez les sujets non obèses. La méta-analyse a révélé

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disorder of glucose metabolism with an increasing prevalence worldwide. Multiple environmental and genetic factors contribute to T2DM development. Genome-wide association studies (GWAS) have reported and replicated nearly 75 susceptibility loci influencing T2DM predisposition and related metabolic traits mostly in Europeans, and some in Africans and Asians, often with unknown functions [1,2]. These include CDKAL1, EXT2, HHEX, IGF2BP2, KCNJ11, SLC30A8 and TCF7L2 [3,4]. Most of these studies involved European and Asian populations, while few data are available for Middle Eastern Arabs [5]. Differences between European- and Asian-derived and Middle Eastern Arab populations, coupled with the diverse ethnic origins of present-day Arabs and heterogeneity of T2DM indicate that the risk imparted by a specific variant may not be identical [5,6] and that diversity in GWAS findings is the norm, not the exception [7,8].

Exostosin-2 (EXT2) is a glycosyltransferase involved in pancreatic development, regulation of insulin synthesis [9] and osteochondroma pathogenesis [10,11]. Several EXT2 polymorphisms — in particular, rs1113132, rs3740878 and rs11037909 — were investigated for their association with T2DM [9,12–14]. An association between these EXT2 variants and T2DM risk was originally reported among French subjects [14]. However, subsequent replication studies showed marginal [9] or no [12,15,16] association between these EXT2 variants and T2DM, indicating ethnic dependency for their association [9,12] and calling for further assessment of the association of EXT polymorphisms with T2DM in different populations.

The present study explored the association of EXT2 rs1113132, rs3740878 and rs11037909 variants with T2DM in 995 Lebanese T2DM patients and 1076 control subjects. Lebanon is located in the Eastern Mediterranean and the modern-day Lebanese population is an admixture of the ancient Phoenician gene pool with Arabian and Western European lineages as a result of Islamic expansion from the Arabian Peninsula in the seventh century and Crusader activity in the 11th–13th centuries, respectively [17]. This is the first study to examine such an association in an Eastern Mediterranean population.

2. Subjects and methods

2.1. Study population

The study group included 995 unrelated T2DM patients who were recruited from outpatient endocrinology clinics in Beirut, Lebanon. T2DM diagnosis was in accordance with the 1999 World Health Organization (WHO) criteria (fasting plasma glucose [FPG] greater than 7.0 mmol/L and/or 2-h plasma glucose greater than 11.1 mmol/L). Neither family history of diabetes nor body mass index (BMI) influenced patient selection. Patients with other types of diabetes or who were diagnosed with T2DM before age 30 years were excluded. The control group included 1076 subjects who were unrelated to any others in the study groups, and had FPG levels lower than 6.1 mmol/L (and 2-h plasma glucose less than 7.8 mmol/L where available) with no family history of diabetes; patients with impaired fasting glucose (IFG), defined as FPG levels at 5.6–6.9 mmol/L, were excluded. Informed consent was obtained from all participants, and the local ethics committees approved the study protocol, which was in accordance with Declaration of Helsinki II guidelines. All subjects were Lebanese Arabs, as non-Arab subjects (Armenians and other minorities) were excluded.

2.2. EXT2 genotyping

The EXT2 gene contains 2299 single nucleotide polymorphisms (SNPs), comprising 21 5’ near gene, seven in 5'-UTR (n = 7), 93 in exons, 14 in 3'-UTR and 12 3’ near gene, with the remainder comprising intronic SNPs (www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=2132). Three SNPs were tested for previous links with T2DM [14] and their frequency in Caucasians. EXT2 genotyping was performed by the allelic discrimination method, using the StepOne Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The TaqMan PCR Assay ID was C_26482681_10 for rs1113132, C_1240647_1 for rs3740878 and C_1240651_20 for rs11037909 in EXT2. In all, 94 duplicate blinded quality-control samples taken from patients and controls were each independently and blindly genotyped to assess reproducibility of the genotyping concordance was >99%. EXT2 genotype frequencies were consistent with the Hardy-Weinberg equilibrium (HWE; Table 1), and the minor allele frequencies (MAFs) obtained were consistent with those of the HapMap CEU sample.

2.3. Statistical analysis

The three SNPs were tested for HWE using the χ² goodness-of-fit test (http://cdsweb01.fhcrc.org/HPlus). Overall power was calculated as the average power of the SNPs genotyped after computing the power for each SNP (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). With α = 0.05, this sample size provided 86% power, assuming a 100% genotype call rate. A pooled effect size was
obtained by meta-analysis (www.metaanalysis.com), assuming a fixed-effects model. Pairwise linkage disequilibrium (LD) values were calculated using SNPStats software (http://bioinfo.iconcologia.net/snpstats). Logistic regression analysis was performed to calculate risk allele-specific \( P \) values, odds ratios (ORs) and 95% confidence intervals (CIs) after controlling for gender, age, BMI, waist–hip ratio and serum triglyceride levels as covariates. \( P \) values were corrected for multiple comparisons using the Bonferroni method.

### 3. Results

#### 3.1. Study subjects

Clinical characteristics of the study participants are presented in Table 1. Gender \( (P = 0.02) \), age at examination \( (P = 0.27) \), total cholesterol \( (P = 0.10) \), high-density lipoprotein (HDL; \( P = 0.48) \) cholesterol and low-density lipoprotein (LDL; \( P = 0.84) \) cholesterol were all comparable between cases and controls. However, mean BMI \( (P = 0.01) \), waist–hip ratio \( (P < 0.001) \) and triglyceride levels \( (P = 0.006) \) were significantly different between patients and controls and therefore were the covariates that were controlled for in the subsequent analyses.

### 3.2. Association studies

Table 2 summarizes the association between \( EXT2 \) SNPs and T2DM in our case-control subjects. Genotypes of rs1113132 \( (P = 0.22) \), rs11037909 \( (P = 0.47) \) and rs3740878 \( (P = 0.11) \) were in HWE among the control population. MAFs of rs11037909 \( (P = 0.028) \) and rs3740878 \( (P = 0.048) \), but not rs1113132 \( (P = 0.84) \), were higher in patients compared with controls. However, this association was lost after applying Bonferroni’s correction for multiple testing. Different associations of the \( EXT2 \) variants with T2DM were seen, depending on BMI. While rs1113132 \( (P = 0.071) \), rs11037909 \( (P = 0.806) \) and rs3740878 \( (P = 0.753) \) were not associated with T2DM in obese subjects (BMI > 30 kg/m\(^2\)), both rs1113132 \( (P = 0.042) \) and rs11037909 \( (P = 0.036) \), but not rs3740878 \( (P = 0.075) \), were associated with T2DM in non-obese subjects. Using the non-carrier genotype as the reference \( (OR = 1.00) \), regression analysis confirmed the association of rs1113132 G/C \( (P = 0.021, OR = 95\% CI = 2.86 (1.17–7.01)) \) and rs11037909 T/C \( (P = 0.033, OR = 95\% CI = 2.69 (1.12–7.26)) \), but not rs3740878, with T2DM in non-obese subjects.

Table 3 is a summary of the results of the association analyses between \( EXT2 \) genotypes in the additive, dominant and recessive genetic models. None of the tested \( EXT2 \) SNPs were associated with T2DM in any of the three models tested. Meta-analyses of our findings as well as those of Moroccan [12] and Tunisian [18] studies revealed a significant association of rs11037909 \( (P = 0.021, OR = 95\% CI = 1.17 (1.00–1.33)) \) and a marginal association of rs3740878 \( (P = 0.052, OR = 95\% CI = 1.14 (0.99–1.33)) \) with T2DM in the fixed model, whereas none of the three \( EXT2 \) variants was associated with T2DM in the random model (Table 4).

#### 3.3. Haplotype analysis

Three-locus (rs1113132/rs11037909/rs3740878) haplotype analysis demonstrated that the majority of haplotype diversity was captured by four haplotypes (GTA, CCG, CTA and GCC) in both the controls (96.7%) and cases (94.8%). Taking the common \( EXT2 \) haplotype as reference \( (OR = 1.00) \), multivariate analysis demonstrated no association between any of the \( EXT2 \) haplotypes with T2DM, not even before correcting for multiple testing (Table 5).
Table 3
Effects of EXT2 genotypes on type 2 diabetes risk according to different genetic models.

<table>
<thead>
<tr>
<th>Genotype combination</th>
<th>Controls distribution</th>
<th>Patients distribution</th>
<th>Additive model OR (95% CI)</th>
<th>Dominant model OR (95% CI)</th>
<th>Recessive model OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1113132 G/G</td>
<td>749 (0.70)</td>
<td>640 (0.64)</td>
<td>0.18 1.00 (reference)</td>
<td>0.11 1.27 (0.95–1.69)</td>
<td>0.73 0.89 (0.47–1.70)</td>
</tr>
<tr>
<td>G/C</td>
<td>265 (0.25)</td>
<td>305 (0.31)</td>
<td>1.33 0.98–1.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>62 (0.06)</td>
<td>50 (0.05)</td>
<td>0.97 0.51–1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs11037909 T/T</td>
<td>745 (0.69)</td>
<td>631 (0.63)</td>
<td>0.46 1.00 (reference)</td>
<td>0.38 1.15 (0.84–1.57)</td>
<td>0.59 0.83 (0.41–1.66)</td>
</tr>
<tr>
<td>T/C</td>
<td>270 (0.25)</td>
<td>296 (0.30)</td>
<td>1.21 0.87–1.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>61 (0.06)</td>
<td>68 (0.07)</td>
<td>0.87 0.43–1.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3740878 A/A</td>
<td>750 (0.70)</td>
<td>644 (0.65)</td>
<td>0.53 1.00 (reference)</td>
<td>0.28 1.19 (0.87–1.62)</td>
<td>0.53 1.26 (0.62–2.59)</td>
</tr>
<tr>
<td>A/G</td>
<td>281 (0.26)</td>
<td>299 (0.30)</td>
<td>1.17 0.84–1.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>45 (0.04)</td>
<td>52 (0.05)</td>
<td>1.32 0.64–2.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Adjusted for gender, age, body mass index, waist–hip ratio and serum triglyceride levels.
b Number of subjects (frequency).

Table 4
Summary of a meta-analysis of three Arab population studies (including the present one).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Fixed model</th>
<th>Random model</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>Z score</td>
<td>P</td>
</tr>
<tr>
<td>rs3740878</td>
<td>1.14 (0.99–1.31)</td>
<td>1.94</td>
</tr>
<tr>
<td>rs1113132</td>
<td>1.05 (0.93–1.17)</td>
<td>0.77</td>
</tr>
<tr>
<td>rs11037909</td>
<td>1.17 (1.02–1.33)</td>
<td>2.30</td>
</tr>
</tbody>
</table>

4. Discussion

Although EXT2 was identified in 2007 as a T2DM susceptibility gene in a large French sample population [14], subsequent studies failed to confirm similar associations in Asians [16,19], African-Americans [15], North Africans [14] and Europeans [14]. The present study found a nominal association between EXT2 SNPs rs1113132, rs11037909 and rs3740878 and T2DM among Lebanese Arabs, which is clearly inconsistent with the previous report by Sladek et al. [14] of European populations. Our present study was the first replication study of the common EXT2 variants in an Eastern Mediterranean Arab (Lebanese) population. As ethnicity is an important factor in genetic association studies [7,8], only Lebanese subjects of Arab origin were included and the controls were matched to patients according to geographical origins to minimize the possibility of population bias.

While rs11037909 and rs3740878 showed marginal associations with T2DM, this was lost after correcting for multiple comparisons. The original study by Sladek et al. [14] found a strong association between rs1113132, rs11037909 and rs3740878 with T2DM in a large (2622 patients, 2900 controls) French population. However, shortly thereafter, Cauchi et al. [12] reported a lack of association between all three EXT2 variants and T2DM in a larger (3295 patients, 3595 controls) French population. Similarly, a separate Chinese study [19] and a large-scale meta-analysis [9] reported marginal/no association of the three EXT2 variants with T2DM. Given the numbers of cases and controls included in these studies, the apparent contradiction does not appear to be due to a lack of power, but is more likely attributable to differences in the selection of the study subjects and perhaps also their ethnic/racial backgrounds.

It is possible that the SNPs used in our study are not causal variants but proxies, and a flip-flop association effect indicating

Table 5
EXT2 haplotype distribution in type 2 diabetes mellitus (T2DM) patients and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls distribution</th>
<th>T2DM cases distribution</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G T A</td>
<td>0.784 ± 0.014</td>
<td>0.757 ± 0.016</td>
<td>0.320</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>C C G</td>
<td>0.147 ± 0.013</td>
<td>0.160 ± 0.017</td>
<td>0.70</td>
<td>1.05 (0.81–1.38)</td>
</tr>
<tr>
<td>C T A</td>
<td>0.022 ± 0.005</td>
<td>0.011 ± 0.009</td>
<td>0.69</td>
<td>0.86 (0.39–1.86)</td>
</tr>
<tr>
<td>G C G</td>
<td>0.014 ± 0.004</td>
<td>0.020 ± 0.011</td>
<td>0.21</td>
<td>1.60 (0.78–3.27)</td>
</tr>
<tr>
<td>C C A</td>
<td>0.008 ± 0.004</td>
<td>0.023 ± 0.011</td>
<td>0.05</td>
<td>1.84 (1.01–3.33)</td>
</tr>
<tr>
<td>G C A</td>
<td>0.013 ± 0.004</td>
<td>0.010 ± 0.011</td>
<td>0.54</td>
<td>0.74 (0.28–1.94)</td>
</tr>
</tbody>
</table>

a rs1113132/rs11037909/rs3740878 determined by maximum likelihood method.
b Fisher’s exact test.
c Haplotype frequency ± SD.
that ‘susceptible’ alleles can change according to the population investigated has also been observed [20,21]. To address this issue, a limited meta-analysis was performed that included only three studies, involving Arab populations in Lebanon (current study), Morocco [12] and Tunisia [18]. While a significant association with T2DM was clearly evident with rs11037909, only a marginal or no association with T2DM was obtained for the other EXT2 variants studied. However, it is noteworthy that the Sladek et al. [14] study reporting a strong association with the three EXT2 variants involved non-obese T2DM patients with a strong family history of T2DM, and cases and controls matched for age, gender and BMI, thereby prompting speculation of a false-positive association result. Yet, the marginal/no association of EXT2 SNPs in the large-scale meta-analysis by Liu et al. [9] does not necessarily support the hypothetical false-positive association claim. Indeed, larger-scale meta-analyses coupled with fine-mapping strategies in populations with multiple ethnic backgrounds are needed to either support or rule out the role of EXT2 variants as a T2DM predisposing gene.

Interestingly, the three tested EXT2 variants were in strong LD (D’ > 0.85, r > 0.82; P < 0.001) and most of the haplotype diversity was captured in four haplotypes (> 94.5%). Also, haplotype analysis failed to identify any susceptible or protective haplotype, thereby further pointing to a lack of association of EXT2 variants with T2DM.

Our present study has demonstrated no association of rs1113132, rs3740878 and rs11037909 EXT2 variants with T2DM. Given the documented lack of association between these EXT2 variants and diabetes in populations of diverse racial backgrounds except for the study by Sladek et al. [14] suggests that EXT2 variants do not constitute T2DM susceptibility genes in Caucasian and non-Caucasian populations.

However, one limitation of our study is that, as only Lebanese subjects of self-reported Arab origin were included, the possibility of population stratification cannot be totally excluded as no ancestry-informative markers were used in our analyses. In addition, the absence of replication does not necessarily imply exclusion, and the possibility of the presence of other SNPs in the EXT2 gene that might reveal genuine associations with T2DM cannot be excluded at this time either, given the lack of fine-mapping of the T2DM-associated LD region in EXT2 with a dense set of SNPs. Thus, further studies are required to confirm the contribution of these and other likely EXT2 variants to T2DM risk.

Disclosure of interest

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

References


