

## T2DM GWAS in the Lebanese population confirms the role of TCF7L2 and CDKAL1 in disease susceptibility

Ghassibe-Sabbagh, Michella; Haber, Marc; Salloum, Angelique K.; Al-Sarraj, Yasser; Akle, Yasmine; Hirbli, Kamal; Romanos, Jihane; Mouzaya, Francis; Gauguier, Dominique; Platt, Daniel E.; El-Shanti, Hatem; Zalloua, Pierre A.

DOI:

[10.1038/srep07351](https://doi.org/10.1038/srep07351)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

### Document Version

Publisher's PDF, also known as Version of record

### Citation for published version (Harvard):

Ghassibe-Sabbagh, M, Haber, M, Salloum, AK, Al-Sarraj, Y, Akle, Y, Hirbli, K, Romanos, J, Mouzaya, F, Gauguier, D, Platt, DE, El-Shanti, H & Zalloua, PA 2014, 'T2DM GWAS in the Lebanese population confirms the role of TCF7L2 and CDKAL1 in disease susceptibility', *Scientific Reports*, vol. 4, 7351.  
<https://doi.org/10.1038/srep07351>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.



OPEN

# T2DM GWAS in the Lebanese population confirms the role of *TCF7L2* and *CDKAL1* in disease susceptibility

SUBJECT AREAS:

DISEASES

GENOME-WIDE ASSOCIATION  
STUDIES

Received

7 January 2014

Accepted

14 November 2014

Published

8 December 2014

Michella Ghassibe-Sabbagh<sup>1</sup>, Marc Haber<sup>1</sup>, Angelique K. Salloum<sup>1</sup>, Yasser Al-Sarraj<sup>2</sup>, Yasmine Akle<sup>3</sup>, Kamal Hirbli<sup>1,4</sup>, Jihane Romanos<sup>1</sup>, Francis Mouzaya<sup>1</sup>, Dominique Gauguier<sup>5</sup>, Daniel E. Platt<sup>6</sup>, Hatem El-Shanti<sup>2,7</sup> & Pierre A. Zalloua<sup>1,8</sup>

<sup>1</sup>Lebanese American University, School of Medicine, Beirut 1102 2801, Lebanon, <sup>2</sup>Shafallah Medical Genetics Center, Doha, Qatar, <sup>3</sup>Centre Hospitalier du Nord-CHN, Zgharta, Lebanon, <sup>4</sup>University Medical Center - Rizk Hospital (UMC-RH), Lebanon, <sup>5</sup>INSERM, UMRS872, Centre de Recherche des Cordeliers, Paris, France, <sup>6</sup>Bioinformatics and Pattern Discovery, IBM T. J. Watson Research Centre, Yorktown Hgts, NY 10598, USA, <sup>7</sup>University of Iowa Carver College of Medicine, Iowa City, <sup>8</sup>Harvard School of Public Health, Boston MA 02215, USA.

Correspondence and requests for materials should be addressed to P.A.Z. (pierre.zalloua@lau.edu.lb)

**Genome-wide association studies (GWAS) of multiple populations with distinctive genetic and lifestyle backgrounds are crucial to the understanding of Type 2 Diabetes Mellitus (T2DM) pathophysiology. We report a GWAS on the genetic basis of T2DM in a 3,286 Lebanese participants. More than 5,000,000 SNPs were directly genotyped or imputed using the 1000 Genomes Project reference panels. We identify genome-wide significant variants in two loci *CDKAL1* and *TCF7L2*, independent of sex, age and BMI, with leading variants rs7766070 (OR = 1.39,  $P = 4.77 \times 10^{-9}$ ) and rs34872471 (OR = 1.35,  $P = 1.01 \times 10^{-8}$ ) respectively. The current study is the first GWAS to find genomic regions implicated in T2DM in the Lebanese population. The results support a central role of *CDKAL1* and *TCF7L2* in T2DM susceptibility in Southwest Asian populations and provide a plausible component for understanding molecular mechanisms involved in the disease.**

**T**ype 2 diabetes mellitus (T2DM) is a chronic metabolic disease with a complex pathogenesis defined by genetic predisposition and environmental factors<sup>1</sup>. In Lebanon, T2DM was evaluated on 8,050 Lebanese cases in 1990. Its prevalence and incidence, similar to the international averages, were 5.0% and 1.5 to 1.7% respectively<sup>2</sup>. In 1992, a study on 436 cases from all over the Lebanese territory gave prevalence of 7 to 8% for T2DM and 10 to 11% for impaired glucose tolerance<sup>3</sup>. In 2005, a study on 3,000 exclusively Lebanese individuals from Greater Beirut showed a prevalence of 11.3% which increased with older age<sup>4</sup>. The combined prevalence of previously and newly diagnosed T2DM was 15.8%. At that time, in the U.S., 6.3% of the population had T2DM: 4.5% diagnosed and 1.8% undiagnosed according to the 2004 National Diabetes Fact Sheet. These results suggest that the prevalence of T2DM in Greater Beirut is relatively high and is increasing among the Lebanese population.

Furthermore, according to the latest figures in the American National Diabetes Statistics Report, 29.1 million children and adults in the United States have diabetes, and 86 million people have prediabetes<sup>5</sup>. The pathogenesis of T2DM is closely associated with a positive family history, male gender, age over 45 years, overweight, hypertension, and abnormal lipid levels. In addition, the genetic contribution to T2DM is well recognized with a total of 91 established associated susceptibility loci<sup>6–17</sup>. The common variants in the reported loci however account for only a small proportion of the heritability of T2DM and the functional role of most of these variants remains far from clear. It is possible that a large number of highly-penetrant but rare T2DM susceptibility genetic variants remain to be identified. Additional genome-wide explorations including whole genome and exome sequencing in well-established groups of patients and controls may unravel these additional important genetic disease contributors which will undoubtedly help us understand the complex mechanisms involved in the development of T2DM.

The prevalence of T2DM is distinctly variable across populations and this variability adds to the disease complexity. This variability could be due to differences in lifestyle factors such as dietary habits, as well as behavioral patterns among populations. Multiple established T2DM susceptibility genetic loci have been identified from previous Genome Wide Association Studies (GWAS) in populations of European and Asian ethnicities<sup>17–19</sup>. It is however, equally important to replicate the behavior of these previously discovered associated



Table 1 | Distribution in the surveyed population of age, gender, BMI, and coronary artery disease by T2DM diagnostic status

	Healthy controls	T2DM patients	Total
Sample size	1,902	1,384	3,286
Age (SD)	62.40 (11.82)	63.62 (9.98)	62.92 (11.1)
Gender (%)			
Female	597 (54)	510 (46)	1107
Male	1305 (60)	874 (40)	2179
BMI (SD)	28.07 (4.54)	28.21 (4.74)	28.13 (4.63)
Coronary artery disease (%)	1300 (56)	1022 (44)	2,322

variants in ethnically different populations and to identify novel predisposing genetic factors that may be stronger in their association than in previously studied populations.

To identify T2DM susceptibility loci in the Lebanese population, we performed a GWAS of more than 5,000,000 SNPs, which were directly genotyped or imputed using the 1000 Genomes Project reference panels. Our study enrolled 1,388 patients and 1,902 non-diabetic subjects with detailed cross-sectional demographic and clinical information. Previously, targeted T2DM susceptibility SNP replication studies have been conducted in the Lebanese population. However, this study seeks to establish a baseline association for T2DM in the Lebanese population and thus it is the first genome-wide scan to find genomic regions implicated in T2DM. We also make use of recent reference data from the 1000 Genomes Project, which was previously shown to identify novel and refined associations for type 2 diabetes mellitus<sup>20,21</sup>.

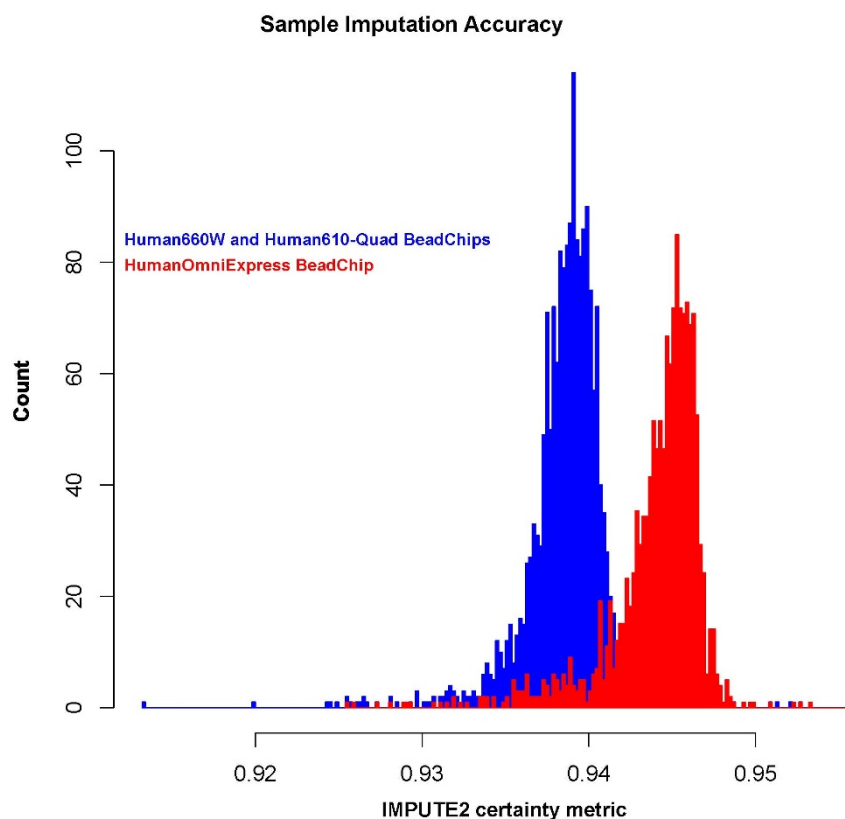
## Results

The study population has a mean age of 62.9 ( $\pm 11.1$ ) (Table 1). The healthy control group has a mean age of 62.40 ( $\pm 11.82$ ), compared to 63.62  $\pm$  (9.98) for the T2DM patients. Sixty-three percent of the

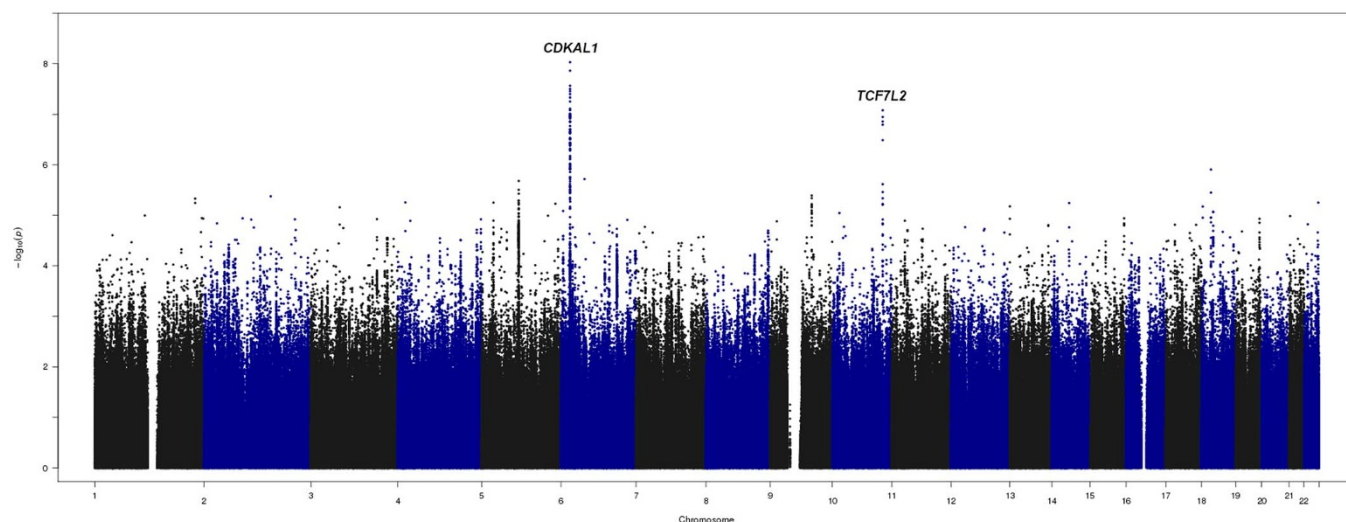
individuals were males with 40.05 percent suffering from T2DM. The mean BMI was of 28.1 ( $\pm 4.6$ ) according to standard measures. 52 samples that failed genotype call rate ( $< 95\%$ )<sup>27</sup> and that showed relatedness (kinship coefficient range  $> 0.0442$ )<sup>28</sup>, were removed.

**Imputation.** 37995922 sites were imputed. The final set included 5891794 SNPs kept after QC (MAF  $> 0.05$ , AIMs removal). The accuracy of the genotype imputation depends on the array SNP coverage and the similarity of haplotypes between the study dataset and the reference panels. The Lebanese population was previously shown to be genetically very close to Europeans<sup>29</sup> that are well represented in the 1000 Genomes Project references. This resulted in high imputation accuracy as shown by Impute2 certainty metric (Figure 1). Figure 1 shows biased imputation confidence depending on chip with the HumanOmniExpress (~700,000 SNPs) having better imputation accuracy compared to the Quad BeadChips (~550,000 SNPs). However, leading principal components reveals no bias regarding population coverage or stratification between chips (FigureS1).

**Association with T2DM.** To map genetic loci associated with T2DM, genotyped and imputed data in 3,286 Lebanese individuals



**Figure 1 | Imputation accuracy.** Per-sample imputation confidence scores between true genotypes and genotypes predicted by imputation, averaged over imputation chunks. Accuracy increases with increased array SNP coverage.



**Figure 2 | Manhattan plot.** Plot shows results of T2DM GWA analysis assuming additive impact in 3286 Lebanese using >5,000,000 genotyped or imputed SNPs. The Y-axis corresponds to the significance of the association ( $-\log_{10} p$ -values). The X-axis represents the physical location of the variant colored by chromosome.

were tested for association with the disease (Figure 2). We identify variations located on chromosomes 4, 6, 9, 10, 12, and 18 that show trends of association with T2DM reaching  $P < 10^{-5}$  under an additive model (Supplementary Table 1). Seven variants located in *CDKAL1* (cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1) reached genome-wide significance ( $P < 5 \times 10^{-8}$ ) with lead signal rs7766070 (OR = 1.38,  $P = 9.37 \times 10^{-9}$ ) (Figure 3, Table 2).

Adjustment for sex, age, and BMI (Table 2) identified 16 additional genome-wide significant variants in *CDKAL1* and *TCF7L2* (Transcription factor 7-like 2) and showed that association of these two loci with T2DM appear to be independent from age and BMI. These variants are also added to Table S1 along with imputation scores.

The genomic control inflation factor ( $\lambda$ ) which compares observed association statistics against the expected distribution, was 1.073 suggesting possible but marginal over-dispersion of the association statistics (Figure 4)<sup>30</sup>.

SNPs of interest that showed trend of association but did not reach genome-wide significance were 12 variants on chromosome 9 (Supplementary Table 1), overlapping a region with microRNA 3910-1 and 3910-2 downstream the gene *ROR2* (receptor tyrosine kinase-like orphan receptor 2).

Three of the SNPs, rs7766070, rs9368222, and rs34872471, identified in Table 2 were significant in DIAGRAM consortium<sup>6,7,9–11,15,17</sup>. One other, rs10440833<sup>15</sup>, was identified but not genome wide significant (Table S2), but which may be of particular interest in Middle Eastern populations<sup>31</sup>. Table S2 lists DIAGRAM consortium study SNPs along with comparisons with our results and imputation confidence scores.

## Discussion

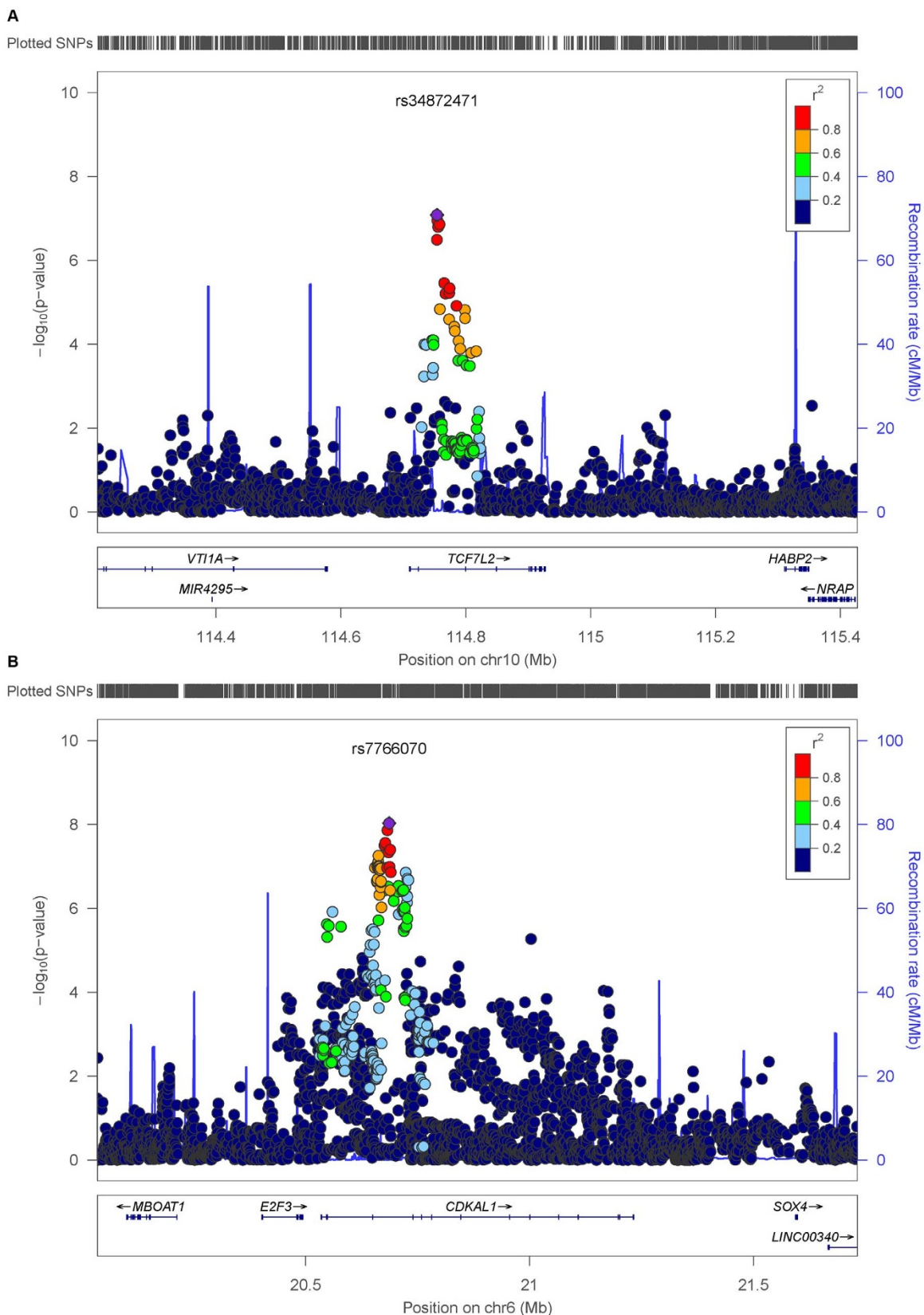
T2DM global prevalence has been steadily increasing over the past 50 years and is today considered a major international health concern. In addition to environmental factors such as diet and lifestyle, genetic susceptibility appears to have substantial role in the etiology of T2DM. Furthermore, T2DM risk alleles show significant disparity in frequencies across worldwide populations. Studies analyzing distribution patterns of genetic diseases found that T2DM risk alleles demonstrated the most extreme differentiation<sup>32</sup>, with population frequencies decreasing from Sub-Saharan Africa, and through Europe to East Asia<sup>32,33</sup>.

The current work contributes to the ongoing worldwide effort to identify the genetic factors that affect the risk of T2DM. We provide analysis in a novel population that is highly admixed with ancestral components from the Middle East, Europe, Central Asia, and sub-Saharan Africa<sup>29</sup>. The risk alleles identified in the Lebanese population could assist in understanding the disparity in T2DM prevalence across worldwide populations.

We report the first genome-wide scan for genetic susceptibility to T2DM in Lebanese subjects. We demonstrate strong association of T2DM with *CDKAL1* and *TCF7L2* genes previously shown to predispose to this condition<sup>15,18,19,24,26,34–37</sup>. A large number of genetic variants have recently been identified to be associated with T2DM in GWASs in Europeans<sup>6,10,15,17,19,25</sup>. The elucidation of the genetic mechanisms responsible for the heterogeneous condition of T2DM in different ethnicities may give important contributions to better understand the complexity of this disease. While some associations are consistent across different ethnic groups<sup>38,39</sup>, some, like the *TCF7L2* variants, have been found to be heterogeneous<sup>6,25</sup>. Our study confirmed genome-wide association of 23 different variants across the *CDKAL1* and *TCF7L2* genes previously reported to play a role in T2DM in different populations<sup>23,40,41</sup>. This association remained significant after adjustment for sex, age, and BMI, suggesting that these risk factors are not likely to be major contributors to the observed association.

After adjusting for age and BMI, most variants in *CDKAL1* and *TCF7L2* showed stronger association to T2DM, indicating that these genes confer susceptibility to T2DM independently from the classical clinical risks. Functional studies of these two genes show their implication in down-regulating blood glucose level. *CDKAL1* encodes a 65 kD protein of unknown function expressed in pancreatic islet and skeletal muscle<sup>26</sup>. *CDKAL1* shows homology with CDK5 regulatory subunit associated protein 1 (*CDK5RAP1*), an inhibitor of CDK5 activation. CDK5 plays a role in the regulation of pancreatic beta cell function by down-regulating insulin expression<sup>42,43</sup>. The *TCL7L2* gene product is a high mobility group (HMG) box-containing transcription factor implicated in blood glucose homeostasis. *TCL7L2* acts through regulation of proglucagon through repression of the proglucagon gene in enteroendocrine cells via the Wnt signaling pathway<sup>44</sup>.

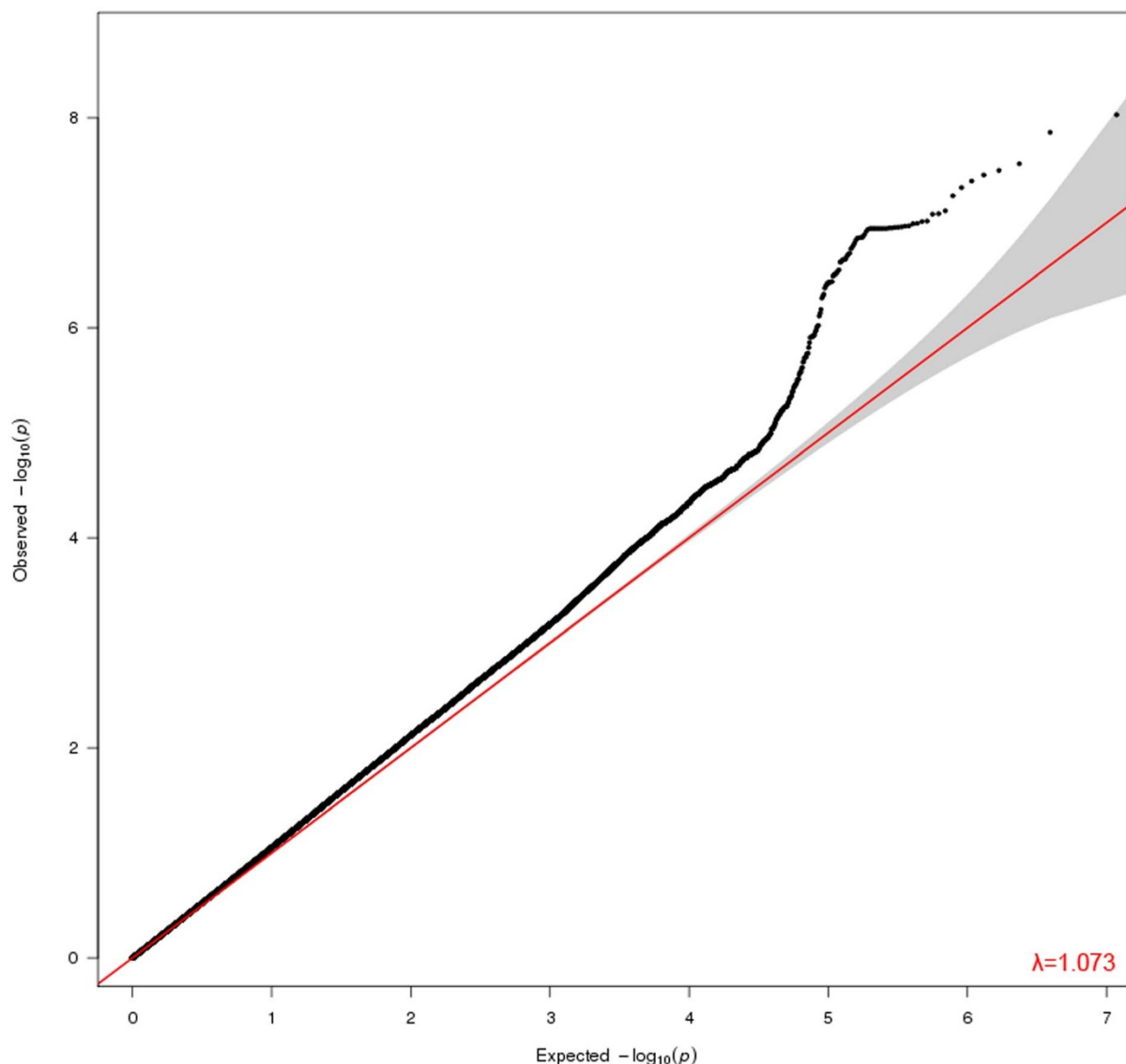
T2DM is a socio-economic burden in Lebanon and genetic studies aim at posing important public health questions in regard to strategies for diagnosis, treatment, and prevention of the disease. Previous replication studies examining a total of 29 SNPs in the Lebanese



**Figure 3 | Regional association plots for the *TCF7L2* (A) and *CDKAL1* (B) genes.** Each regional plot shows the chromosomal position (hg 19) of SNPs in the specific region against  $-\log_{10} p$  values estimated assuming additive impact. The SNP with the highest association signal at each locus is shown as a purple star; the other SNPs are colored according to the extent of LD with that SNP. Estimated recombination rates from the 1000 Genomes Project European population (release March 2012) are shown as light blue lines.

Table 2 | T2DM GWA results showing SNPs that reached genome-wide significance ( $<5 \times 10^{-8}$ ) in the initial testing phase or after conditioning for sex, age, and BMI

SNP	Imputation	Chr	gene	MAF	Additive model			Adjusted for sex			Adjusted for age			Adjusted for BMI			Adjusted for sex, age, and BMI		
					OR	95%CI	p-value	OR	95%CI	p-value	OR	95%CI	p-value	OR	95%CI	p-value	OR	95%CI	p-value
rs7766070	imputed	6	CDKAL1	0.29	1.38	1.24-1.54	9.37E-09	1.38	1.24-1.54	1.71E-08	1.38	1.24-1.54	4.64E-09	1.39	1.24-1.55	6.83E-09	1.39	1.25-1.55	4.77E-09
rs9348441	imputed	6	CDKAL1	0.29	1.38	1.24-1.53	1.38E-08	1.38	1.24-1.53	2.33E-08	1.38	1.24-1.53	7.16E-09	1.39	1.24-1.54	9.26E-09	1.39	1.24-1.55	6.38E-09
rs35261542	imputed	6	CDKAL1	0.29	1.38	1.23-1.53	2.75E-08	1.38	1.23-1.53	4.58E-08	1.38	1.23-1.53	1.56E-08	1.39	1.24-1.55	1.93E-08	1.39	1.24-1.55	1.45E-08
rs7451008	imputed	6	CDKAL1	0.29	1.37	1.23-1.53	3.18E-08	1.37	1.23-1.53	5.16E-08	1.37	1.23-1.53	1.91E-08	1.39	1.24-1.55	1.63E-08	1.39	1.24-1.55	1.31E-08
rs1569699	genotyped	6	CDKAL1	0.38	1.33	1.21-1.48	3.51E-08	1.33	1.21-1.48	7.11E-08	1.34	1.21-1.48	1.74E-08	1.35	1.22-1.49	1.85E-08	1.35	1.22-1.49	1.49E-08
rs9368222	imputed	6	CDKAL1	0.29	1.36	1.22-1.52	4.02E-08	1.36	1.22-1.52	6.46E-08	1.37	1.23-1.52	2.18E-08	1.38	1.23-1.53	2.49E-08	1.38	1.23-1.53	1.65E-08
rs9350271	imputed	6	CDKAL1	0.37	1.33	1.2-1.47	4.64E-08	1.33	1.2-1.47	9.50E-08	1.33	1.2-1.47	2.18E-08	1.34	1.21-1.49	3.40E-08	1.34	1.21-1.49	2.59E-08
rs10946396	imputed	6	CDKAL1	0.38	1.33	1.2-1.47	5.56E-08	1.33	1.2-1.47	1.04E-07	1.33	1.2-1.47	2.49E-08	1.34	1.21-1.48	5.23E-08	1.34	1.21-1.48	4.08E-08
rs6456369	imputed	6	CDKAL1	0.38	1.33	1.2-1.47	7.72E-08	1.33	1.2-1.47	1.36E-07	1.33	1.2-1.47	3.38E-08	1.33	1.2-1.48	7.33E-08	1.33	1.2-1.48	5.20E-08
rs6456368	imputed	6	CDKAL1	0.31	1.35	1.21-1.5	8.23E-08	1.35	1.21-1.5	1.32E-07	1.35	1.21-1.5	3.88E-08	1.35	1.21-1.5	9.80E-08	1.35	1.21-1.5	6.91E-08
rs7754840	imputed	6	CDKAL1	0.31	1.34	1.21-1.49	9.67E-08	1.34	1.21-1.49	1.48E-07	1.34	1.21-1.49	4.45E-08	1.35	1.21-1.5	1.02E-07	1.35	1.21-1.5	6.71E-08
rs4710940	imputed	6	CDKAL1	0.38	1.32	1.2-1.46	9.77E-08	1.32	1.2-1.46	1.83E-07	1.33	1.2-1.47	4.35E-08	1.33	1.2-1.47	8.27E-08	1.33	1.2-1.48	6.28E-08
rs35456723	imputed	6	CDKAL1	0.3	1.35	1.21-1.5	1.02E-07	1.35	1.21-1.5	1.66E-07	1.35	1.21-1.5	4.86E-08	1.35	1.21-1.5	1.03E-07	1.35	1.21-1.5	7.44E-08
rs9295474	imputed	6	CDKAL1	0.31	1.35	1.21-1.5	1.07E-07	1.35	1.21-1.5	1.69E-07	1.35	1.21-1.5	5.19E-08	1.36	1.22-1.51	6.71E-08	1.36	1.22-1.51	4.51E-08
rs7756992	genotyped	6	CDKAL1	0.3	1.34	1.21-1.49	1.08E-07	1.34	1.21-1.49	1.78E-07	1.34	1.21-1.5	5.14E-08	1.35	1.21-1.51	6.63E-08	1.35	1.22-1.51	4.33E-08
rs6456367	imputed	6	CDKAL1	0.31	1.34	1.21-1.49	1.10E-07	1.34	1.21-1.49	1.71E-07	1.34	1.21-1.49	4.94E-08	1.35	1.21-1.5	1.09E-07	1.35	1.21-1.5	7.07E-08
chr6:20660689:D	imputed	6	CDKAL1	0.37	1.33	1.2-1.47	1.11E-07	1.33	1.2-1.47	1.96E-07	1.33	1.2-1.47	4.96E-08	1.33	1.2-1.48	1.03E-07	1.33	1.2-1.48	7.54E-08
rs7752780	imputed	6	CDKAL1	0.31	1.34	1.21-1.49	1.11E-07	1.34	1.21-1.49	1.71E-07	1.34	1.21-1.49	4.93E-08	1.34	1.21-1.5	1.15E-07	1.35	1.21-1.5	7.31E-08
rs34872471	imputed	10	TCF7L2	0.4	1.32	1.19-1.46	8.30E-08	1.32	1.19-1.46	1.51E-07	1.32	1.2-1.46	4.80E-08	1.35	1.22-1.49	9.91E-09	1.35	1.22-1.5	1.01E-08
rs35198068	imputed	10	TCF7L2	0.4	1.31	1.19-1.45	1.12E-07	1.31	1.19-1.45	2.03E-07	1.32	1.19-1.46	6.61E-08	1.34	1.21-1.49	1.38E-08	1.35	1.22-1.49	1.42E-08
rs7903146	genotyped	10	TCF7L2	0.4	1.31	1.19-1.45	1.39E-07	1.31	1.19-1.45	2.44E-07	1.31	1.19-1.45	8.38E-08	1.34	1.21-1.48	1.74E-08	1.34	1.21-1.49	1.79E-08
rs4506565	imputed	10	TCF7L2	0.41	1.31	1.18-1.44	1.60E-07	1.31	1.18-1.44	2.70E-07	1.31	1.19-1.45	9.64E-08	1.34	1.21-1.48	2.00E-08	1.34	1.21-1.48	1.96E-08
rs7901695	genotyped	10	TCF7L2	0.41	1.3	1.18-1.43	3.23E-07	1.3	1.18-1.43	5.53E-07	1.3	1.18-1.44	2.03E-07	1.33	1.2-1.47	4.35E-08	1.33	1.2-1.47	4.50E-08



**Figure 4 | Quantile-Quantile plot of the GWAS results.** Plot compares observed  $-\log_{10} p$  values of the tested SNPs on the vertical axis to expected  $-\log_{10} p$  values under the null hypothesis on the horizontal axis. The genomic control ratio was 1.073, indicating the lack of strong effect of systematic error such as population stratification.

population have identified variants associated with T2DM in *COL8A1*, *KCNQ1*, *ALX4*, *HNF1*<sup>45</sup> (n = 2071), *CDKAL1*<sup>50</sup> (n = 1422), and *TCF7L2*<sup>46,47</sup> (n = 1610).

Our genome-wide analysis of more than 5,000,000 SNPs in the largest T2DM study to date in the region has only identified variations in the *CDKAL1* and *TCF7L2* genes to be genome wide significantly associated with the disease.

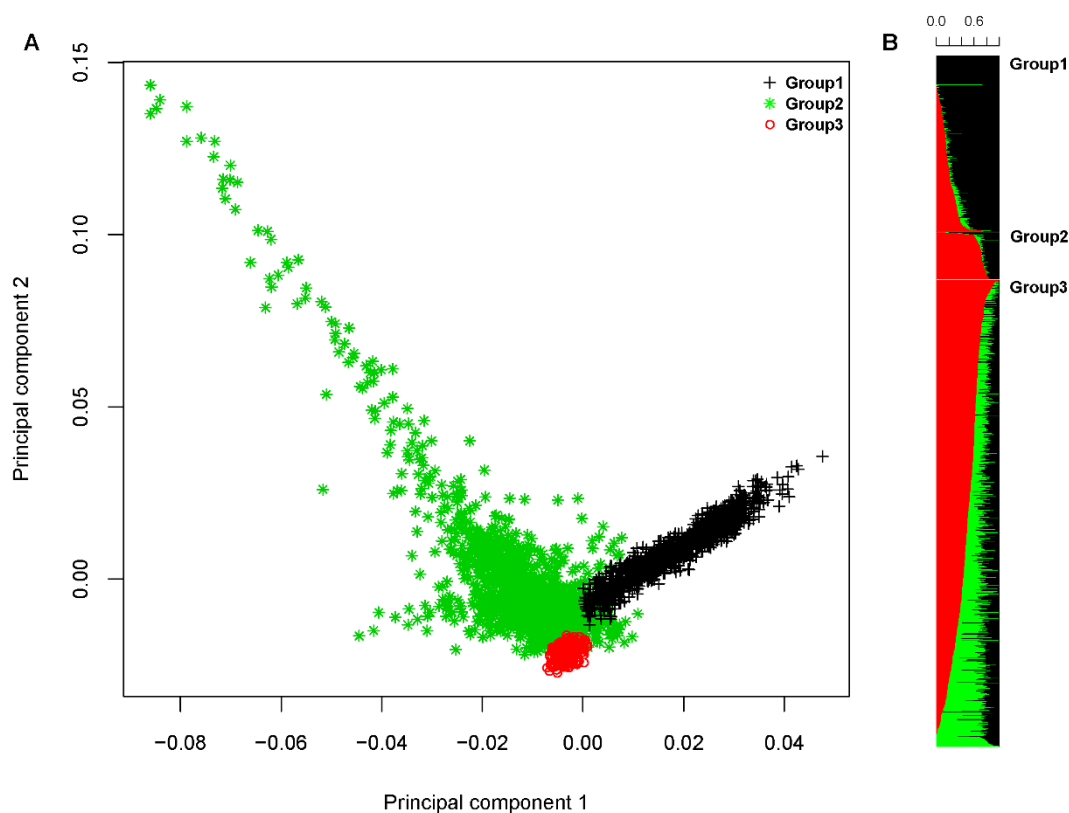
Lebanon represents a Levantine region with higher European affinity than other parts of the Middle East<sup>48</sup>, and therefore potentially fills in the map between SNPs identified in European populations contrasted with other Middle Eastern populations. Further, since T2DM is an emerging issue within the Middle East, these populations may differentially highlight some variants distinct from other regions. Even accepting relatively low power compared to that available in meta-studies, the moderately small number of replicated GWS significant SNPs in our study is less than expected, especially given the affinity of Lebanon with other European populations<sup>48</sup>.

Our results contribute to ongoing efforts to identify and confirm risk alleles associated with T2DM. Mapping disease alleles in different populations can provide important perspectives in disease dia-

gnosis and steer functional studies to provide better understanding of the pathogenesis of T2DM.

## Methods

**Study Participants.** The Lebanese study participants were all of Lebanese origin and were recruited to this study in three phases. A first recruitment campaign, conducted with the collaboration of the Lebanese University Medical Center, led to the recruitment of 506 subjects from the suburbs of Beirut, the capital of Lebanon. In a second campaign conducted in North Lebanon, 492 subjects were successfully recruited. The 2,292 remaining participants were recruited as part of the Functional Genomic Diagnostic Tools for Coronary Artery Disease project initiative (FGENTCARD) from the Rafic Hariri University Hospital, and the “Centre Hospitalier du Nord” in Lebanon<sup>49,50</sup>. Research and methods were carried out in compliance with the Helsinki Declaration and with the approval of the LAU institutional review board and local ethics committees on human research (Reference number SMPZ08072010-4). All participants signed an informed consent and data and blood samples were obtained from each individual. By taking part in the two recruitment campaigns, participants (1) answered a detailed questionnaire, (2) gave a blood sample for DNA analysis and (3) gave a blood sample for HbA1C, fasting blood glucose (FBS), and lipid profile measures after 12 hours fasting. For the remaining FGENTCARD 2,292 participants, a detailed questionnaire was duly filled, a blood sample was obtained for DNA and metabolites analysis and annotations were coded from medical charts for data such as laboratory tests, prescribed medications, and presence of clinical conditions. Table 1 describes clinical and demographic



**Figure 5 | Population structure in the Lebanese.** A) Principal component analysis shows an orthogonal population structure. Model-based clustering using mclust identifies three groups similar to previously reported stratification in the Lebanese. B) Estimation of individual ancestry using ADMIXTURE at  $K = 3$  shows three groups with disparate proportions of ancestral allele frequencies.

characteristics of the study participants<sup>49</sup>. Previously reported<sup>51</sup> analysis of these subjects showed association with recruitment for questionnaire variables, such as “exercise level,” “smoking usage”. However, blood panel associations showed very consistent odds ratios for T2DM and coronary artery disease risk independently of recruitment.

**Selection of Patients and Controls.** For the 998 participants selected through the two recruitment campaigns, an HbA1C of 48 mmol/mol (6.5%) was used as the cut-off point for diagnosing T2DM in line with the World Health Organization definition of type 2 diabetes mellitus diagnosis<sup>52</sup>. For the 2,292 participants from the FGENTCARD project, T2DM was diagnosed by an ascertained physician supported by subjects HbA1C levels and or their two-hour plasma glucose concentration after an oral glucose tolerance test as documented in their medical records. For the control dataset, 85.23% of the participants were  $\geq 50$  years old and have no history of T2DM. Body mass index (BMI) was calculated according to standard measurements. Selection criteria for DNA analysis were based on the complete availability of the genotyping data and the T2DM-relevant characteristics, resulting in the selection of 3,286 subjects.

**Genotyping and imputation.** DNA was extracted using a standard phenol-chloroform extraction procedure. Samples were genotyped using Illumina HumanOmniExpress-12V1-1 Multi-use (623 controls, 839 cases,  $\sim 700,000$  SNPs) and Illumina Human610-660W Quad BeadChips (1279 controls, 545 cases,  $\sim 550,000$  SNPs). Plink<sup>27</sup> was used for data management and quality control keeping 3,286 samples with call rate  $\geq 95\%$ , SNPs call rate  $\geq 98\%$ , Hardy-Weinberg  $p > 10^{-6}$ , MAF  $\geq 1\%$ . Genotype data was converted to NCBI genome build 37 using LiftOver (<http://genome.ucsc.edu/cgi-bin/hgLiftOver>). Chromosomes were phased with SHAPEIT<sup>53</sup> using the 1000 Genomes Phase I haplotypes<sup>54</sup> provided on the IMPUTE2 website (1000 Genomes Phase I integrated haplotypes (produced using SHAPEIT2), September 2013) based on sequence data for 1,092 TGP samples from release 20110521. IMPUTE2<sup>55</sup> was employed to impute genotypes in blocks of  $\sim 5$  Mb using all populations in the 1000 Genomes Phase I data. Accuracy of imputation was measured using IMPUTE2’s information metric “info”.

**Statistical analysis.** GWAS can be confounded by population stratification. The most common approach to correct for population stratification is to include the principal components from the PCA as covariates. However, there is no solid method to determine how many components to include and selection is usually subjective with inconclusive results. Correction for stratification becomes even more complicated in populations like the Lebanese where genetic structure is recent and not driven by the

usual factors of distance and geography<sup>29</sup>. Furthermore, previous studies have shown that historical migrations to Lebanon have potentially created disease-associated population structures that are similar to those in the source populations<sup>22,56</sup>. In this study, we use insights from our previous studies on population stratifications in the Lebanese to avoid type 1 errors in the GWAS.

We apply a method that uses available 1000 Genomes Project reference panels to impute a resolved ancestry dataset<sup>29</sup> as well as the current T2DM cohort to the same SNP coverage resolution. We next use two model-based clustering methods to unambiguously classify subjects into groups (Figure 5):

- 1- mclust (<http://www.stat.washington.edu/mclust/>) uses model-based hierarchical clustering and Bayesian Information Criterion to classify samples into related groups.
- 2- ADMIXTURE<sup>57</sup> assigns individuals in a model-based manner into ancestral populations using maximum likelihood estimates.

The two methods identified similar population structures and assigned samples into three ancestral groups (Figure 5) similar to previous ancestry reports on the Lebanese<sup>29</sup>. We next proceed by extracting 192,310 ancestry informative markers (AIMs) between the groups and exclude it from the GWA analysis. We have also tested our method by using the identified ancestry groups as covariates in the GWAS and obtained identical results.

Association tests were performed using SNPTEST v2.4<sup>58</sup>, employing frequentist association tests and taking into account uncertainty in imputed genotypes (calling threshold 0.9). At each SNP, an additive, dominant, or recessive model was tested versus a model of no association. Association tests were also conditioned on age, sex, and BMI to test for a genetic effect over and above that explained by these covariates. Chip type and enrollment were also both included in a separate analysis; results showed correlated but no genome-wide significant SNPs. Further, a PCA painted by chip type (Figure S1) shows no significant bias. LocusZoom<sup>59</sup> was used to plot regional association results from the genome-wide association scans.

1. Stumvoll, M., Goldstein, B. J. & van Haefen, T. W. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* **365**, 1333–46 (2005).
2. Hirbly, K. I., Aboujaoude, J. H., Ghorra, F. S. & Barakat-el-Khoury, W. M. [Prevalence and incidence of diabetes mellitus in Lebanon]. *Diabete Metab* **16**, 479–83 (1990).
3. Hirbly, K. I., Gerges, T. A., Karam, V. J. & Saikaly, J. A. [The estimation of the prevalence of diabetes mellitus in Lebanon]. *J Med Liban* **40**, 22–30 (1992).





4. Hirbli, K. I. *et al.* Prevalence of diabetes in greater Beirut. *Diabetes Care* **28**, 1262 (2005).
5. Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services (2014) (Date of access:03/10/2014). Available from: <http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf>.
6. Cho, Y. S. *et al.* Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. *Nat Genet* **44**, 67–72 (2012).
7. Dupuis, J. *et al.* New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* **42**, 105–116 (2010).
8. Kong, A. *et al.* Parental origin of sequence variants associated with complex diseases. *Nature* **462**, 868–874 (2009).
9. Kooner, J. S. *et al.* Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. *Nat Genet* **43**, 984–990 (2011).
10. Morris, A. P. *et al.* Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet* **44**, 981–990 (2012).
11. Qi, L. *et al.* Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. *Hum Mol Genet* **19**, 2706–15 (2010).
12. Scott, R. A. *et al.* Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet* **44**, 991–1005 (2012).
13. Shu, X. O. *et al.* Identification of new genetic risk variants for type 2 diabetes. *PLoS Genet* **6**, e1001127 (2010).
14. Tsai, F. J. *et al.* A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. *PLoS Genet* **6**, e1000847 (2010).
15. Voight, B. F. *et al.* Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* **42**, 579–89 (2010).
16. Yamauchi, T. *et al.* A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. *Nat Genet* **42**, 864–8 (2010).
17. Zeggini, E. *et al.* Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* **40**, 638–45 (2008).
18. Scott, L. J. *et al.* A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* **316**, 1341–5 (2007).
19. Unoki, H. *et al.* SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. *Nat Genet* **40**, 1098–102 (2008).
20. Hara, K. *et al.* Genome-wide association study identifies three novel loci for type 2 diabetes. *Hum Mol Genet* **23**, 239–46 (2013).
21. Huang, J., Ellinghaus, D., Franke, A., Howie, B. & Li, Y. 1000 Genomes-based imputation identifies novel and refined associations for the Wellcome Trust Case Control Consortium phase 1 Data. *Eur J Hum Genet* **20**, 801–5 (2012).
22. Haber, M. *et al.* Y-chromosome R-M343 African lineages and sickle cell disease reveal structured assimilation in Lebanon. *J Hum Genet* **56**, 29–33 (2011).
23. Peng, S. *et al.* TCF7L2 gene polymorphisms and type 2 diabetes risk: a comprehensive and updated meta-analysis involving 121, 174 subjects. *Mutagenesis* **28**, 25–37 (2013).
24. Saxena, R. *et al.* Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. *Am J Hum Genet* **90**, 410–25 (2012).
25. Saxena, R. *et al.* Genome-wide association study identifies a novel locus contributing to type 2 diabetes susceptibility in Sikhs of Punjabi origin from India. *Diabetes* **62**, 1746–55 (2013).
26. Zeggini, E. *et al.* Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* **316**, 1336–41 (2007).
27. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559–75 (2007).
28. Manichaikul, A. *et al.* Robust relationship inference in genome-wide association studies. *Bioinformatics* **26**, 2867–73 (2010).
29. Haber, M. *et al.* Genome-wide diversity in the levant reveals recent structuring by culture. *PLoS Genet* **9**, e1003316 (2013).
30. Devlin, B., Roeder, K. & Wasserman, L. Genomic control, a new approach to genetic-based association studies. *Theor Popul Biol* **60**, 155–66 (2001).
31. Al-Daghri, N. M. *et al.* Association between type 2 diabetes mellitus-related SNP variants and obesity traits in a Saudi population. *Mol Biol Rep* **41**, 1731–40 (2014).
32. Chen, R. *et al.* Type 2 diabetes risk alleles demonstrate extreme directional differentiation among human populations, compared to other diseases. *PLoS Genet* **8**, e1002621 (2012).
33. Corona, E. *et al.* Analysis of the genetic basis of disease in the context of worldwide human relationships and migration. *PLoS Genet* **9**, e1003447 (2013).
34. Consortium, T. W. T. C. C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–78 (2007).
35. Okada, Y. *et al.* Common variants at CDKAL1 and KLF9 are associated with body mass index in east Asian populations. *Nat Genet* **44**, 302–6 (2012).
36. Perry, J. R. *et al.* Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. *PLoS Genet* **8**, e1002741 (2012).
37. Stancakova, A. *et al.* Single-nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. *J Clin Endocrinol Metab* **93**, 1924–30 (2008).
38. Haiman, C. A. *et al.* Consistent directions of effect for established type 2 diabetes risk variants across populations: the population architecture using Genomics and Epidemiology (PAGE) Consortium. *Diabetes* **61**, 1642–7 (2012).
39. Rong, R. *et al.* Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians. *Diabetes* **58**, 478–88 (2009).
40. Horikawa, Y. *et al.* Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. *J Clin Endocrinol Metab* **93**, 3136–41 (2008).
41. Palmer, N. D. *et al.* Resequencing and analysis of variation in the TCF7L2 gene in African Americans suggests that SNP rs7903146 is the causal diabetes susceptibility variant. *Diabetes* **60**, 662–8 (2011).
42. Ubeda, M., Rukstalis, J. M. & Habener, J. F. Inhibition of cyclin-dependent kinase 5 activity protects pancreatic beta cells from glucotoxicity. *J Biol Chem* **281**, 28858–64 (2006).
43. Wei, F. Y. *et al.* Cdk5-dependent regulation of glucose-stimulated insulin secretion. *Nat Med* **11**, 1104–8 (2005).
44. Yi, F., Brubaker, P. L. & Jin, T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. *J Biol Chem* **280**, 1457–64 (2005).
45. Almawi, W. Y. *et al.* A replication study of 19 GWAS-validated type 2 diabetes at-risk variants in the Lebanese population. *Diabetes Res Clin Pract* **102**, 117–22 (2013).
46. Nemr, R. *et al.* Replication study of common variants in CDKAL1 and CDKN2A/2B genes associated with type 2 diabetes in Lebanese Arab population. *Diabetes Res Clin Pract* **95**, e37–40 (2012).
47. Nemr, R. *et al.* Transcription factor-7-like 2 gene variants are strongly associated with type 2 diabetes in Lebanese subjects. *Diabetes Res Clin Pract* **98**, e23–7 (2012).
48. Badro, D. A. *et al.* Y-chromosome and mtDNA genetics reveal significant contrasts in affinities of modern Middle Eastern populations with European and African populations. *PLoS One* **8**, e54616 (2013).
49. Saade, S. *et al.* Large scale association analysis identifies three susceptibility loci for coronary artery disease. *PLoS One* **6**, e29427 (2011).
50. Youhanna, S. *et al.* Parental consanguinity and family history of coronary artery disease strongly predict early stenosis. *Atherosclerosis* **212**, 559–63 (2010).
51. Platt, D. E. *et al.* Circulating lipid levels and risk of coronary artery disease in a large group of patients undergoing coronary angiography. *J Thromb Thrombolysis* (2014) Advance online publication. DOI 10.1007/s11239-014-1069-2
52. Colagiuri, S. Glycated haemoglobin (HbA1c) for the diagnosis of diabetes mellitus—practical implications. *Diabetes Res Clin Pract* **93**, 312–3 (2011).
53. Delaneau, O., Zagury, J. F. & Marchini, J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat Methods* **10**, 5–6 (2013).
54. Abecasis, G. R. *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012).
55. Howie, B. N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* **5**, e1000529 (2009).
56. Haber, M. *et al.* mtDNA lineages reveal coronary artery disease-associated structures in the Lebanese population. *Ann Hum Genet* **76**, 1–8 (2012).
57. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* **19**, 1655–64 (2009).
58. Marchini, J. & Howie, B. Genotype imputation for genome-wide association studies. *Nat Rev Genet* **11**, 499–511 (2010).
59. Pruim, R. J. *et al.* LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* **26**, 2336–7 (2010).

## Acknowledgments

We thank the patients for agreeing to participate in the study. We thank Dr. Bouchra Douaiby and Mrs. Hana Sbeite for helping in the campaigns. We thank the dispensaire de la maison maronite de soins et d'hébergement- Ain El Remmaneh, Ehdn Governmental Hospital - Ehdn, Caritas Liban - Dahr el Ain, Lebanese Red Cross - Amioun, Saint Joseph dispensary - Zgharta, and dispensary of Kfarhabou -Donniah for hosting the campaigns. This publication was made possible by a grant from the Qatar National Research Fund under its National Priorities Research program (NPRP) award number NPRP 09-215-3-049. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Qatar National Research Fund.

## Author contributions

Conceived and designed the experiments: M.G.S., M.H., H.E.S., D.E.P. and P.A.Z. Performed the statistical analysis: M.H. Analyzed the data: M.G.S., M.H., A.K.S., D.E.P., H.E.S. and P.A.Z. Contributed reagents/materials/genotyping: Y.A.S., Y.A., K.H., J.R., F.M., D.G. and H.E.S. Wrote the paper: M.G.S., M.H., D.E.P. and P.A.Z. with help from all co-authors.

## Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>



**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Ghassibe-Sabbagh, M. *et al.* T2DM GWAS in the Lebanese population confirms the role of *TCF7L2* and *CDKAL1* in disease susceptibility. *Sci. Rep.* 4, 7351; DOI:10.1038/srep07351 (2014).



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>