

***TCF7L2* is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis**

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Received: 28 January 2007 / Revised: 27 March 2007 / Accepted: 30 March 2007
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Abstract *TCF7L2* variants have been consistently associated with type 2 diabetes (T2D) in populations of different ethnic descent. Among them, the rs7903146 T allele is probably the best proxy to evaluate the effect of this gene

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on T2D risk in additional ethnic groups. In the present study, we investigated the association between the *TCF7L2* rs7903146 polymorphism and T2D in Moroccans (406 normoglycemic individuals and 504 T2D subjects) and in white Austrians (1,075 normoglycemic individuals and 486 T2D subjects). Then, we systematically reviewed the association of this single nucleotide polymorphism (SNP) with T2D risk in a meta-analysis, combining our data with

data from previous studies. The allelic odds ratios (ORs) for T2D were 1.56 [1.29–1.89] ($p=2.9\times 10^{-6}$) and 1.52 [1.29–1.78] ($p=3.0\times 10^{-7}$) in Moroccans and Austrians, respectively. No heterogeneity was found between these two different populations by Woolf test ($\chi^2=0.04$, $df=1$, $p=0.84$). We found 28 original published association studies dealing with the *TCF7L2* rs7903146 polymorphism in T2D. A meta-analysis was then performed on 29,195 control subjects and 17,202 cases. No heterogeneity in genotypic distribution was found (Woolf test: $\chi^2=31.5$, $df=26$, $p=0.21$; Higgins statistic: $I^2=14.1\%$). A Mantel–Haenszel procedure was then performed to provide a pooled odds ratio (OR) of 1.46 [1.42–1.51] ($p=5.4\times 10^{-140}$). No publication bias was detected, using the conservative Egger’s regression asymmetry test ($t=-1.6$, $df=25$, $p=0.11$). Compared to any other gene variants previously confirmed by meta-analysis, *TCF7L2* can be distinguished by its tremendous reproducibility of association with T2D and its OR twice as high. In the near future, large-scale genome-wide association studies will fully extend the genome coverage, potentially delivering other common diabetes-susceptibility genes like *TCF7L2*.

Introduction

TCF7L2 rs7903146 T allele has been consistently associated with type 2 diabetes (T2D) in individuals of European [1–14], Asian [7, 15–17], and African descent [7, 18], so far. Despite comprehensive genotyping efforts across this gene locus [1, 2, 4, 18], this single nucleotide polymorphism (SNP) always showed the strongest association with T2D. Furthermore, Helgason et al. [18] reported that the rs7903146 T allele is probably the ancestral allele and concluded that if this allele is not itself the causative variant, then the unidentified functional variant it tags is likely to lie outside the screened locus. Thus, genotyping this polymorphism is probably the best proxy to evaluate the effect of this gene on T2D risk in additional ethnic groups, and combining data related to this SNP is likely to be sufficient for a meta-analysis of the global contribution of the gene on T2D. In the present study, we examined the association between the *TCF7L2* rs7903146 polymorphism and T2D in two additional populations of different ethnic descents, Central Europeans and, for the first time, North Africans. Then, we systematically reviewed the association of this polymorphism with T2D risk in a meta-analysis combining our data with those from previous studies.

Results

The contribution of the rs7903146 T allele to T2D was assessed in white Austrians (1,075 normoglycemic individ-

uals and 486 T2D subjects) [19], and in Moroccans (406 normoglycemic individuals and 504 T2D subjects). Clinical characteristics and allelic distributions of both populations are reported in Tables 1 and 2. All genotypic distributions were in Hardy–Weinberg equilibrium. The allelic odds ratios (ORs) for T2D were 1.56[1.29–1.89] ($p=2.9\times 10^{-6}$) and 1.52[1.29–1.78] ($p=3.0\times 10^{-7}$) in Moroccans and Austrians, respectively (Table 2). No heterogeneity was found between these two different populations by Woolf test ($\chi^2=0.04$, $df=1$, $p=0.84$). The genetic models were found to be multiplicative in Moroccans ($p=4.1\times 10^{-5}$) and in Austrians ($p=3.2\times 10^{-7}$), rather than departing from linearity ($p=0.13$ and $p=0.19$, respectively).

We found 28 original published association studies dealing with the *TCF7L2* rs7903146 polymorphism in T2D. Unfortunately, the data available on Mexican-American population were not detailed enough to include them in the present analysis [14]. From these studies, a total of 27,705 control individuals and 16,200 subjects with T2D have been analyzed for disease association between T2D and the T allele variant. If we include our own study cohorts (Austrians and Moroccans), the overall total would add up to 29,195 control subjects and 17,202 cases. Considering that all publications were consistent with a multiplicative model of inheritance, the relative risk for T2D was only estimated by allelic odds ratios. No heterogeneity in genotypic distribution was found (Woolf test: $\chi^2=31.5$, $df=26$, $p=0.21$; Higgins statistic: $I^2=14.1\%$).

A Mantel–Haenszel procedure was then performed to provide a pooled odds ratio of 1.46[1.42–1.51] ($p=5.4\times 10^{-140}$) (Fig. 1). No publication bias was detected, using the conservative Egger’s regression asymmetry test ($t=-1.6$, $df=25$, $p=0.11$).

Several other genes have been previously associated with T2D by meta-analysis [20–29]. *KCNJ11* and *PPARG* have reached genome-wide association levels of significance and the role of other genes have been under debate.

Table 1 Clinical characteristics (means±SD) of the T2D case-control studies

Status	Moroccan		Austrian	
	Control	T2D	Control	T2D
<i>N</i>	406	504	1,075	486
Sex ratio (male/female)	121/285	156/348	725/350	285/201
Age at examination (years)	55±12	58±11	51.5±6.0	56.5±9.6
Age at diagnosis (years)	na	51±12	na	49.3±9.5
BMI (kg/m ²)	27.2±5.3	28.0±4.7	26.4±4.0	30.7±6.3

Data presented as means±SD

na Not available

Table 2 Genotypic distributions of rs7903146 among Moroccan and Austrian populations

Parameters	Moroccan (control/T2D)	Austrian (control/T2D)
C/C	176/140	555/200
C/T	185/277	432/208
T/T	54/99	88/78
Hwe (control)	0.67	0.76
HWE (T2D)	0.08	0.07
MAF% (control)	35.3	28.3
MAF% (T2D)	46	37.4
Allelic OR [95% CI]	1.56 [1.92–1.89]	1.52 [1.29–1.78]
<i>p</i> value	2.9×10^{-6}	3.0×10^{-7}

HWE: *p* value for Hardy–Weinberg Equilibrium

The *TCF7L2* gene can be distinguished by its tremendous reproducibility of association with T2D and its OR twice as high (Fig. 2).

Discussion

We positively replicated the association of *TCF7L2* variation and T2D in two populations of different ethnic descent, Central Europeans and, for the first time, North Africans. The most striking result is the very stable relative

risk (around 50%) conferred by the rs7903146 T allele in these two geographically, ethnically, and environmentally diverse populations. The meta-analysis of 27 different studies confirms this finding with a resulting global OR of 1.46 [1.42–1.51], suggesting that in any tested human population the effect of *TCF7L2* is very similar. The absence of heterogeneity between studies is also indicative of a universal contribution of this gene to T2D, the population-attributable risk being only driven by the prevalence of the T allele in a specific ethnic group. This situation is unique as previous candidate genes for T2D have always shown some degree of discrepancy between populations [20–29]. *TCF7L2* has moved rapidly from a novel positional candidate gene to a reference gene for T2D susceptibility [1, 30]. In most ethnic groups, except for Eastern Asians [16, 17], a highly frequent T allele (ranging from 18–35% for controls to 22–45% for cases) offers the possibility to get enough statistical power for association studies [18]. Consequently, almost all published case-control studies were able to detect an association with T2D, except for those with few participants or without clear ethnic belonging [4, 7]. However, even among Caucasian populations there are substantial differences in T allele frequency, e.g., 17% difference between Finns [2] and Moroccans (this study).

So far, few other SNPs have been reproducibly associated with T2D [20–29]. The comparison between meta-

Fig. 1 Association of the *TCF7L2* rs7903146 T allele with T2D: Data are shown for allelic odds ratios based on allele counts. *MAF* stands for minor allele frequency in controls. For each study, the point estimate is given by a *square* whose height is inversely proportional to the standard error of the estimate and the extent of the 95% around the estimate is given by the *horizontal line*. The summary odds ratio is drawn as a *diamond* with horizontal limits at the confidence limits and width inversely proportional to its standard error. In this meta-analysis, we were able to compare 29,195 control individuals with 17,202 subjects with T2D. No heterogeneity in genotypic distribution was found (Woolf test: $\chi^2=31.5$, $df=26$, $p=0.21$; Higgins statistic: $I^2=14.1\%$). No publication bias was detected, using the conservative Egger’s regression asymmetry test ($t=-1.6$, $df=25$, $p=0.11$). A Mantel-Haenszel (fixed effects) procedure was then performed to provide a pooled odds ratio

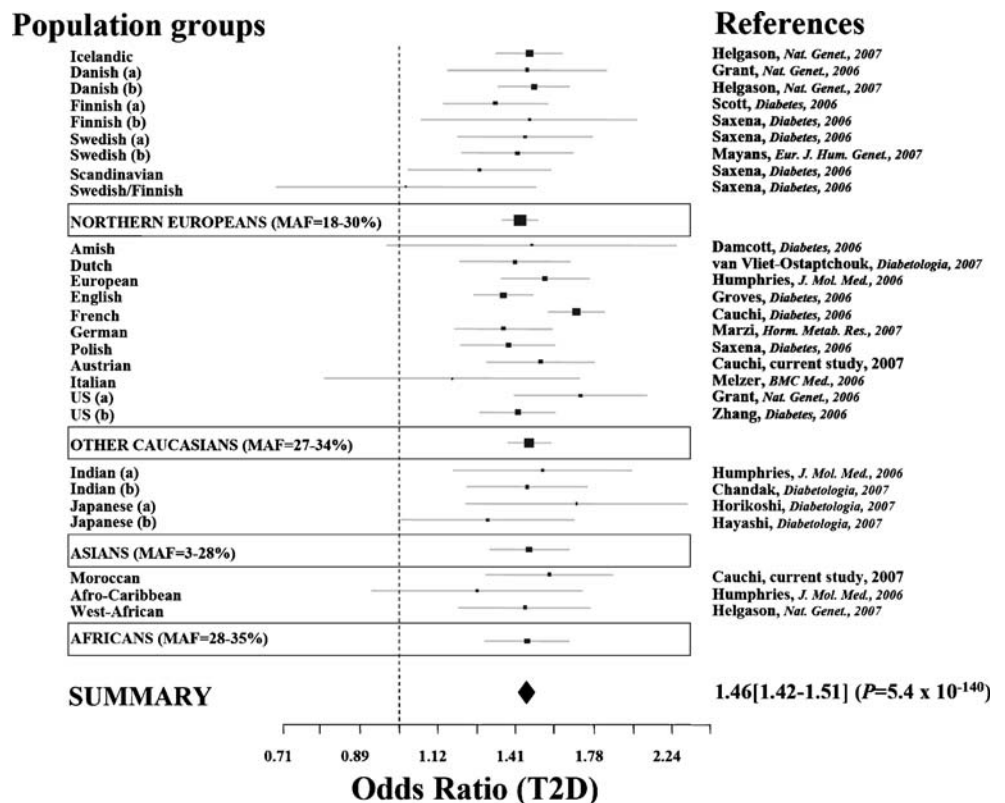
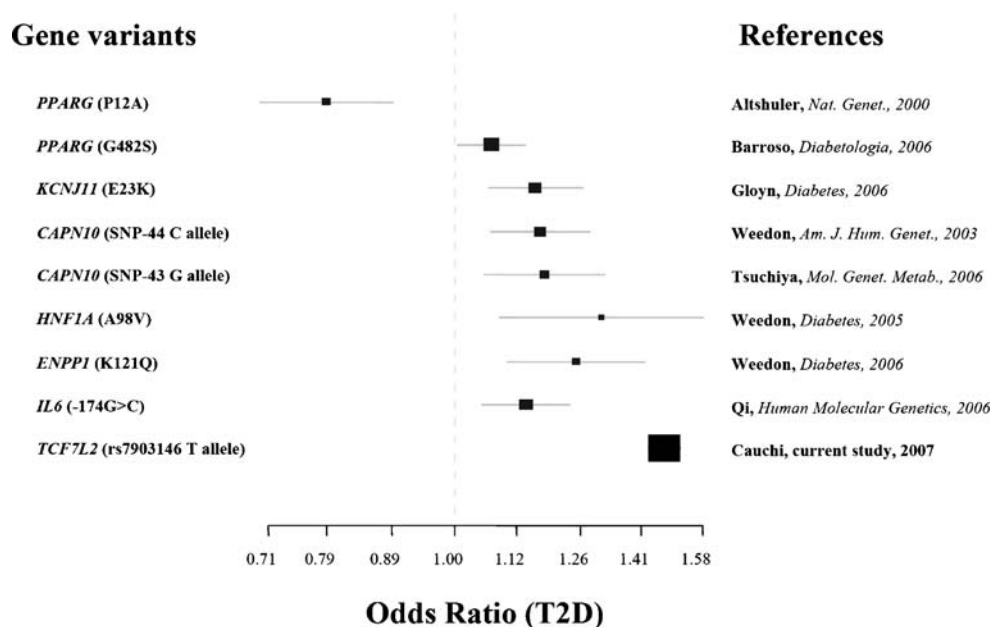


Fig. 2 *TCF7L2*, the most reproducible risk for T2D by a gene variant so far: data are shown for allelic odds ratios based on meta-analyses previously published. The 95% CI for each meta-analysis is represented by a horizontal line. The square's area is proportional to the statistical weight of each meta-analysis



analyses (shown in Fig. 2) clearly illustrates that the magnitude of the *TCF7L2* effect is much higher than any other confirmed T2D candidate. The individual effects of the other variants are modest, ranging from 10 to 30%. Interestingly, no major interactions with the T allele have been found to strongly modulate T2D susceptibility, even if body mass index [5, 7, 9, 16, 18], gender [10], drugs [9], or lifestyle interventions [9] may modulate *TCF7L2* effects. No functional significance has been attributed to the *TCF7L2* T allele so far, which is also different from most T2D SNPs listed in Fig. 2. In rodents, the Wnt/ β -catenin signaling pathway is important for the development of pancreas [31, 32], but the pathophysiology of the *TCF7L2*-associated T2D remains to be clarified in humans.

The binding of transcription factors and alternative splicing events should be studied in the intronic region where the T allele is located. Recent theoretical studies have emphasized that as few as 20 susceptibility variants on the scale of those in *TCF7L2* may suffice to explain as much as 50% of the burden of the disease [33]. In the near future, large-scale genome-wide association data will fully extend the genome coverage, potentially delivering other common diabetes-susceptibility genes like *TCF7L2* [34].

Materials and methods

Subjects Two populations with different ethnic backgrounds were analyzed: white Austrians from central Europe [19] and Moroccans from North Africa.

Moroccan subjects were recruited by the Faculty of Medicine (Fes) and were subject to a standardized clinical

examination at the Hassan II Hospital. Inclusion criteria for cases were: (1) T2D according to 1997 American Diabetes Association (ADA) criteria; (2) family history of diabetes in first degree relatives; (3) BMI <30 kg/m². Inclusion criteria for controls were: (1) age at examination over 45 yr.; (2) normal fasting glucose according to 1997 ADA criteria; (3) BMI <27 kg/m².

Austrian subjects with T2D were recruited from diabetes outpatient clinics of the Landeskliniken Salzburg and the Hallein Hospital. Patients who were <73 years of age and <63 years of age at diagnosis were included. Participants of the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (Saphir) who were not using hypoglycemic medications and had fasting blood glucose levels <110 mg/dl served as control subjects. Study populations comprised only white Europeans, mainly of Bavarian or Austrian-German descent, living in the same geographic region.

The main clinical characteristics were reported in Table 1. The genetic study was approved by local Ethical Committees and informed consent was obtained from all participants. Participants were considered as normoglycemic controls when their fasting glucose concentration was lower than 6.1 mmol/l.

Genotyping methods High-throughput genotyping for the rs7903146 variant was performed using the TaqMan[®] SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). The polymerase chain reaction (PCR) primers and TaqMan probes were designed by Primer Express and optimized according to the manufacturer's protocol. There was a 98% genotyping success rate and the genotyping error rate was assessed by sequencing 384 control and 384

T2D individuals and by re-genotyping a random 10% sample. No difference was found with the first genotyping results, thus the genotyping error rate was 0%.

Statistical methods Tests for deviation from Hardy–Weinberg equilibrium (HWE) and for association were performed with the De Finetti program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). All *P* values were two-tailed. Meta-analysis was performed using the packages “rmeta” and “meta” of the R-Project (<http://www.r-project.org>). The Woolf test was first applied to test the genotypic heterogeneity between studied groups [35], then the Mantel–Haenszel procedure was performed to provide a pooled odds ratio with a 95% confidence interval. We performed the Higgins statistic (I²) to quantify the amount of between-study variability in effect attributable to true heterogeneity rather than chance [36]. We also used the Egger’s regression method to test for publication bias [37].

To assess whether the model of inheritance was multiplicative or departing from linearity, we applied a logistic regression test with two variables corresponding to genotypes v1 (coded 0, 1, 2), reflecting a linear increase in risk, and v2 (coded 0, 1, 0), reflecting a departure from linearity. Different meta-analyses of T2D association studies are discussed in Fig. 2: *PPARG* [20, 21], *KCNJ11* [22, 23], *CAPN10* [24, 25], *HNF1A* [26], *ENPP1* [27], and *IL6* [28, 29]. We only reported studies with allelic odds ratios, based on diverse ethnic groups. SPSS 14.1 software (SPSS, Chicago, IL, USA) was used for general statistical analyses.

Acknowledgments This work was partly supported by the French Governmental “Agence Nationale de la Recherche”, by the French-Moroccan convention “CNRST-CNRS”, and the charities: “Association Française des Diabétiques” and “Programme national de recherche sur le diabète” and “Association des diabétiques de la Wilaya de Fès”. We thank Marianne Deweider and Frederic Allegaert for the DNA bank management and Stefan Gaget for his help on phenotype databases. We are indebted to all subjects who participated to this study.

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