***TCF7L2* single nucleotide polymorphisms, cardiovascular disease and all-cause mortality: the Atherosclerosis Risk in Communities (ARIC) study**

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Abstract

Aims/hypothesis We hypothesised that *TCF7L2* single nucleotide polymorphisms (SNPs) are associated with cardiovascular disease (CVD) and that the associations differ in diabetic and non-diabetic persons.

Methods Our analysis included black and white participants from the Atherosclerosis Risk in Communities study who were free of prevalent CVD at baseline and had been genotyped for rs7903146, rs12255372, rs7901695, rs11196205 and rs7895340 ($n=13,369$). Cox proportional hazard regression was used to estimate the associations between polymorphisms and incident events; logistic and linear regression were used for associations with baseline risk factor levels.

Results *TCF7L2* SNPs were not significantly associated with incident coronary heart disease, ischaemic stroke, CVD, prevalent peripheral artery disease (PAD) or all-cause mortality in the full cohort or when stratified by race.

Conclusions/interpretation In the whole cohort, *TCF7L2* SNPs were not associated with incident CVD, all-cause mortality or prevalent PAD. This result suggests that the

increased health risk associated with rs7903146 genotype is specific to diabetes.

Keywords All-cause mortality · Cardiovascular disease · Coronary heart disease · Diabetes · Peripheral artery disease · Stroke · *TCF7L2* · Transcription factor 7-like 2

Abbreviations

ARIC	Atherosclerosis Risk in Communities
CVD	cardiovascular disease
IMT	intima–media thickness
PAD	peripheral artery disease
SNP	single nucleotide polymorphism
TCF-4	transcription factor 4

Introduction

Diabetes is a major risk factor for stroke, peripheral artery disease (PAD) and CHD. Polymorphisms of *TCF7L2* have been associated with diabetes in numerous studies [1–3] and the magnitude of risk conferred by *TCF7L2* variants is greater than for any previously described common variant. The single nucleotide polymorphism (SNP) rs7903146 is the most highly associated of the known variants and is associated with a 40% increased risk of diabetes per allele [4]. The gene *TCF7L2* is located on chromosome 10 and encodes the transcription factor 4 (TCF-4). Interestingly, a specific role for TCF-4 has been shown in the process of vascular remodelling. Moreover, transcriptional activation of TCF-4 turns on the nuclear factor- κ B signalling pathway, which regulates inflammatory signalling pathways [5]. Data from the MONICA/KORA Surveys showed that the T allele of rs7903146 was inversely associated with homeostasis

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model assessment of per cent beta cell function and fasting insulin, suggesting a role in pancreatic beta cell function [3]. Thus, depleting islet cells of *TCF7L2* in culture resulted in significant increases in beta cell apoptosis and decreases in proliferation and glucose-stimulated insulin secretion [6]. We therefore hypothesised that *TCF7L2* SNPs would be associated with cardiovascular disease (CVD) in the Atherosclerosis Risk in Communities (ARIC) Study. Furthermore, we investigated whether the associations between *TCF7L2* genotypes and CVD differ in ARIC participants with and without diabetes.

Methods

The ARIC study is a prospective cohort study of 15,792 participants investigating the development of atherosclerosis and described in detail elsewhere [7]. Written informed consent was obtained from each study participant prior to data collection at each exam. For the present analysis, individuals were excluded on the basis of the following criteria: race other than black or white ($n=48$); black participants from centres with small numbers ($n=55$); prevalent CHD ($n=763$); missing data for prevalent CHD ($n=339$); prevalent stroke ($n=264$); and missing genotype data for *TCF7L2* SNP rs7903146 ($n=954$). The final analysis sample included 13,369 participants. In addition, individuals for whom ankle-brachial index values were missing were excluded ($n=457$) from the investigation of associations between *TCF7L2* and PAD. Ascertainment and

standardised case definitions for PAD [8], CHD [9], stroke [10], carotid intima-media thickness (IMT) [11], mortality [12] and diabetes [13] have been described elsewhere as indicated. Five *TCF7L2* SNPs (rs7903146, rs12255372, rs7901695, rs11196205 and rs7895340) were genotyped on stored DNA using the TaqMan system (Applied Biosystems, Foster City, CA, USA). Some samples (5%) were re-genotyped for quality control and 726 ARIC participants were genotyped in duplicate. The percentage of agreement was 96% and simple kappa coefficient was 0.93, indicating good genotype quality. All data were analysed with SAS, version 9 (SAS Institute, Cary, NC, USA). We tested for Hardy–Weinberg equilibrium using the χ^2 goodness of fit test. Cox proportional hazard regression was used to estimate the associations between SNPs and incident events; logistic and linear regression were used for baseline prevalence measures. We had 80% power to detect a relative risk of 1.17 for incident CHD with an alpha level at $p=0.05$.

Results

The study population included 27% black individuals and 57% women, with a mean population age of 54 years. All five SNP genotype frequencies were in Hardy–Weinberg equilibrium. The following results focus on rs7903146. Baseline diabetes, current smoking, life-time smoking exposure (pack-years) and BMI (kg/m^2) differed by genotype. For the CC, CT and TT genotypes, respectively, the baseline prevalence of diabetes was 9.4, 12.2 and 14.2%

Table 1 Numbers of events and HRs of incident CVD and all-cause mortality per T allele of rs7903146, ARIC 1987–2004

	Black individuals				White individuals				All			
	Minimally adjusted ^a		Fully adjusted ^b		Minimally adjusted ^a		Fully adjusted ^b		Minimally adjusted ^a		Fully adjusted ^b	
	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)
CHD	389	1.12 (0.96–1.3)	374	1.09 (0.93–1.28)	1,338	1.01 (0.93–1.10)	1,326	1.01 (0.93–1.09)	1,727	1.04 (0.96–1.11)	1,700	1.02 (0.95–1.1)
CHD, diabetes	122	1.11 (0.85–1.45)	119	1.04 (0.79–1.36)	263	1.19 (0.99–1.4)	260	1.21 (1.01–1.44)	385	1.16 (1.0–1.35)	379	1.14 (0.98–1.33)
CHD, no diabetes	202	1.06 (0.86–1.32)	195	1.07 (0.86–1.33)	925	0.93 (0.84–1.03)	917	0.92 (0.83–1.02)	1,127	0.95 (0.87–1.04)	1,112	0.94 (0.86–1.04)
Ischaemic stroke	235	1.12 (0.92–1.36)	221	1.14 (0.93–1.40)	314	0.99 (0.83–1.17)	311	0.97 (0.82–1.16)	549	1.04 (0.91–1.18)	532	1.04 (0.91–1.18)
CVD	594	1.10 (0.98–1.25)	567	1.10 (0.98–1.25)	1,594	1.0 (0.93–1.08)	1,578	0.99 (0.92–1.07)	2,188	1.03 (0.96–1.10)	2,145	1.02 (0.95–1.08)
All-cause mortality	757	1.01 (0.91–1.13)	726	0.99 (0.89–1.11)	1,354	1.03 (0.95–1.12)	1,331	1.00 (0.92–1.08)	2,111	1.03 (0.96–1.09)	2,057	1.00 (0.93–1.07)

^a Adjusted for age and sex; race in the combined analysis

^b Minimally adjusted variables+BMI, smoking status, pack-years

($p < 0.0001$) and the percentage of current smokers was 25, 26 and 28% ($p = 0.03$). The T allele was associated with lower mean BMI (CC=27.8, CT=27.5, TT=27.4 kg/m², $p = 0.03$) and more pack-years of smoking (CC=14.7, CT=15.2 and TT=16.3, $p = 0.05$). There was no difference by genotype for age, race, sex, current drinking, ethanol intake, waist-to-hip ratio, total cholesterol, HDL- and LDL-cholesterol, triacylglycerol or systolic and diastolic blood pressure.

The T allele of rs7903146 was not significantly associated with incident CHD, ischaemic stroke or CVD, or all-cause mortality in the full cohort or when stratified by race (Table 1). In whites with prevalent diabetes, the T allele was associated with incident CHD, but only in the model adjusted for age, sex, BMI and smoking status (HR 1.21, $p = 0.04$). We found no significant association of this allele with prevalent PAD or IMT (data not shown).

Discussion

We hypothesised that *TCF7L2* SNPs would be associated with increased risk of CVD and all-cause mortality given the association of these variants with diabetes. In the whole cohort, rs7903146 was not associated with incident CVD, all-cause mortality or prevalent PAD. The T allele of rs7903146 was associated with smoking and inversely associated with BMI in the whole cohort, and in a subset of whites with prevalent diabetes, the same allele was associated with incident CHD. Investigators of the Go-Darts Study of over 6,500 European participants reported that the T allele was associated with increased HbA_{1c} in both patient and control participants. They also reported that the T allele was over-represented in individuals requiring insulin treatment and under-represented in patients managed by diet alone, suggesting that variants of *TCF7L2* may be associated with disease severity and therapeutic efficacy [14]. Therefore, a reasonable hypothesis is that the small increase in risk of CHD seen in our study may be the result of suboptimal or ineffective treatment of diabetes in the earlier onset cases. However, the increased risk observed in the white subgroup was not seen in the corresponding black subgroup (HR 1.04, 95% CI 0.79–1.36). While the association may be spurious, given that this was one of several subgroup analyses, the inconsistency between races may result from the smaller sample size in this racial group. Among those with prevalent diabetes, we had 80% power to detect a relative risk of 1.54 and 1.78 for incident CHD in white and black participants, respectively. Clearly, replication in a larger and possibly younger diabetic population is needed to confirm or refute this finding.

In their entirety, these results suggest that while there may be a slightly increased risk of CHD in patients with early-onset diabetes, the increased health risk associated with rs7903146 genotype is specific to diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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